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A.M.A. ARCHIVES OF

INTERNAL MEDICINE

Fiftieth Anniversary of the Archives
Paul S. Rhoads

Ulcerative Colitis—A Challenge
Joseph B. Kirsner

An "Autoimmune" Reaction Against Human Tissue Antigens in Certain Acute and Chronic Diseases
I. Serological Investigations
D. Carleton Gajdusek
II. Clinical Correlations
Ian R. Mackay and D. Carleton Gajdusek

Adenovirus Vaccine
M. R. Hilleman

Occurrence of Adenovirus Infections in Civilian Populations
William S. Jordan Jr.

The Special Problem of Rheumatic Heart Disease in Pregnant Women
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The Electromyogram in Myxedema
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Double Mitral Valve
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Scleroderma Associated with Osteopoikilosis
Gerald Weissmann

The Laboratory Diagnosis of Pancreatic Disease
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Stokes-Adams Disease
J. K. Lewis

Contributions of Basic Science to Modern Medicine

Vitamin B_6 in Internal Medicine
Leo Wayne, John J. Will, Ben I. Friedman, Louis S. Becker, and Richard W. Vilter

Essential Fatty Acids and Atherosclerosis
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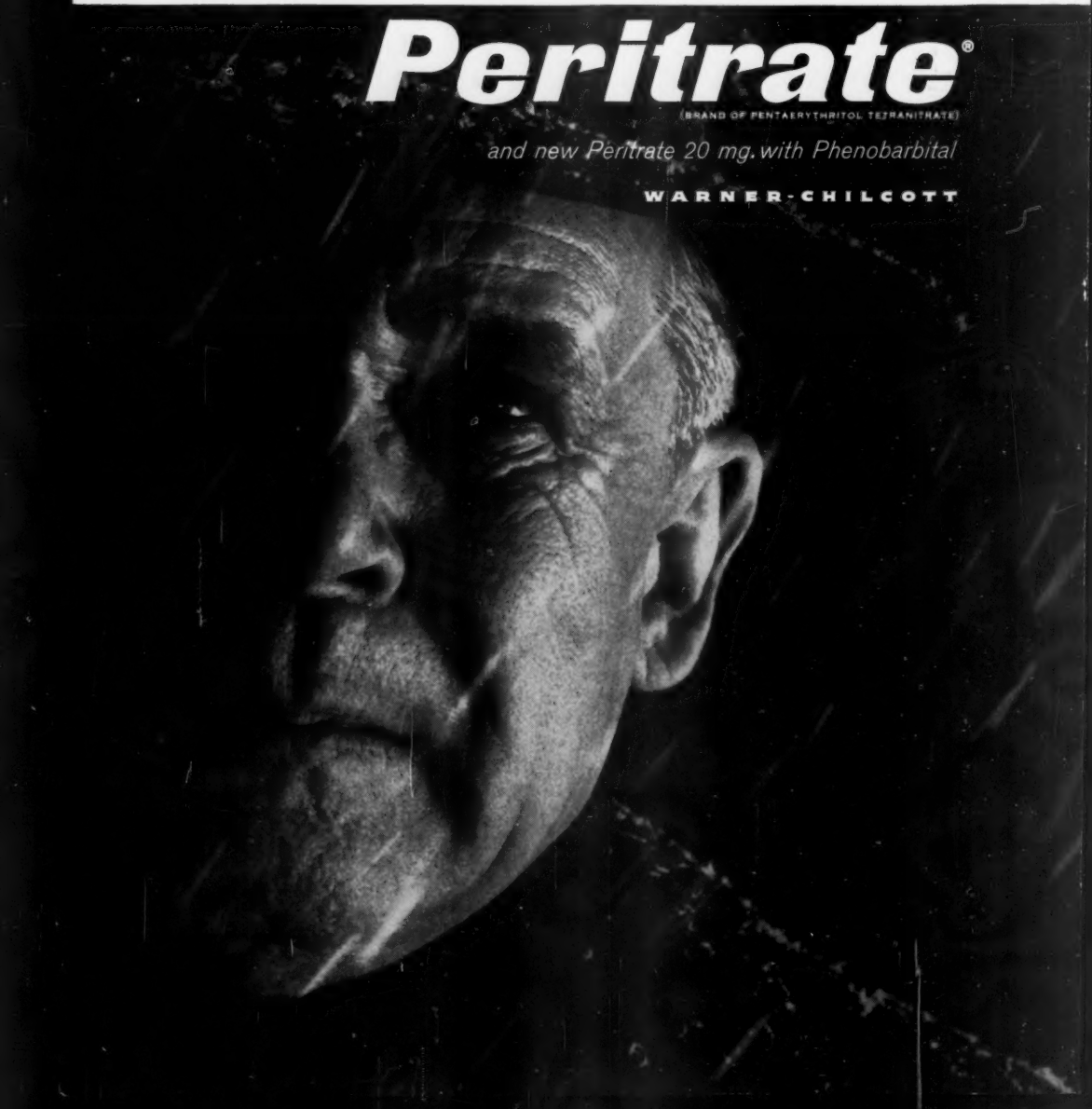

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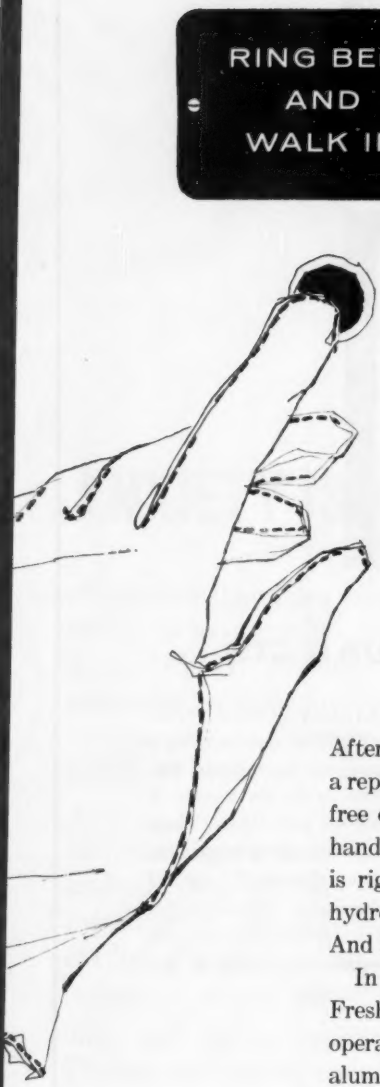

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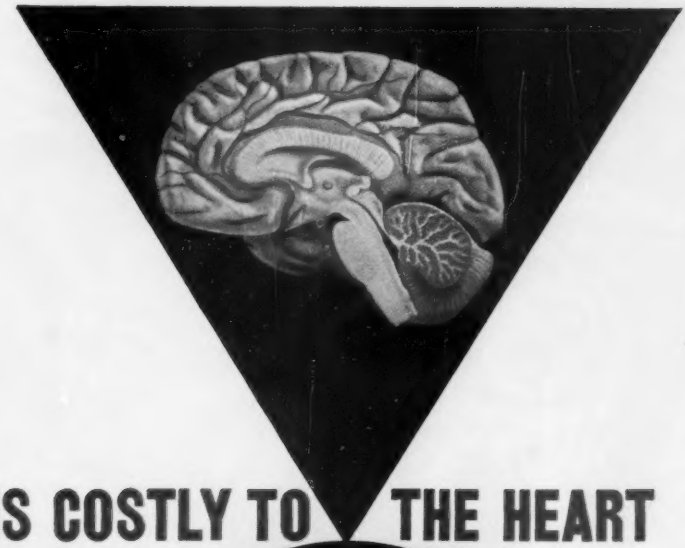

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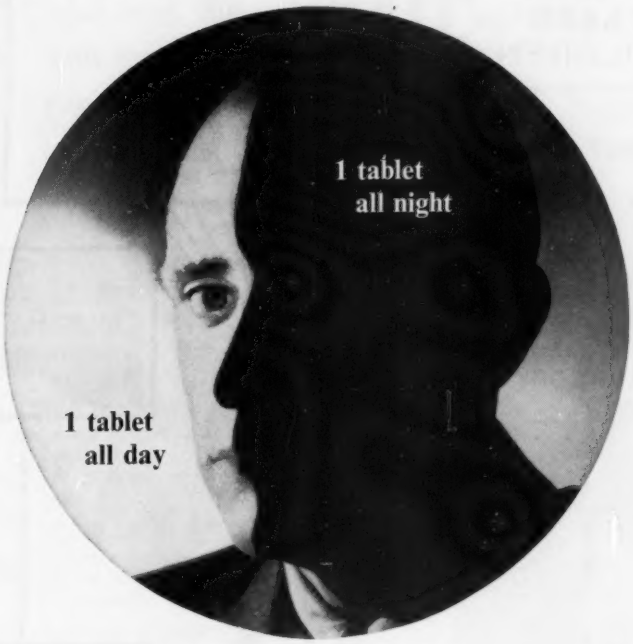

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[1]Fuller, H. L. and Kassel, L. E.: Antibiotic Medicine and Clinical Therapy, 3:322, October 1956.

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1. Warter, P. J.: J. M. Soc. New Jersey 54:7, 1957.
2. Tillis, H. H.: Am. Pract. & Digest Treat. 8:932, 1957.

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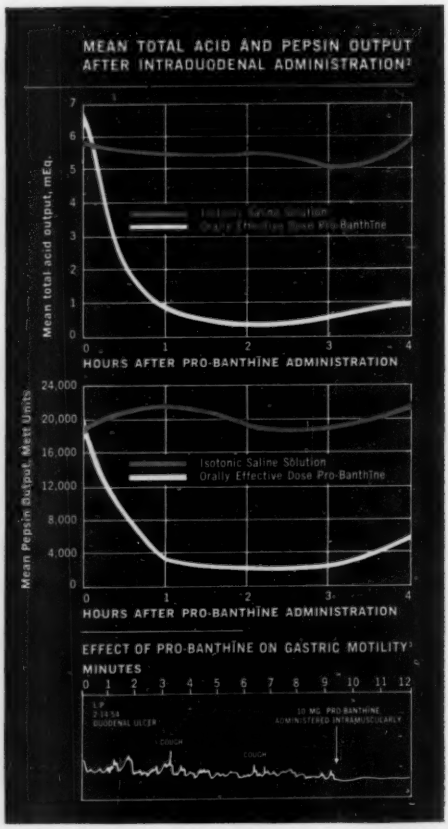

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2. Sun, D. C. H., and Shay, H.: Optimal Effective Dose of Anticholinergic Drug in Peptic Ulcer Therapy, Arch. Int. Med. *97*:442 (April) 1956.
3. Lichstein, J.; Morehouse, M. G., and Osmon, K. L.: Pro-Banthīne in the Treatment of Peptic Ulcer, Am. J. M. Sc. *232*:156 (Aug.) 1956.

SEARLE

Editorials

Fiftieth Anniversary of the Archives

Plans for the Future

With the present issue the A. M. A. ARCHIVES OF INTERNAL MEDICINE reaches its fiftieth year of continuous publication. It is the oldest of the specialty journals of the American Medical Association and was, at the time of its inception, the second periodical in this country to be devoted exclusively to internal medicine.

In 1907, in answer to a petition signed by many leaders of American medicine of the era, the board of trustees of the American Medical Association appointed a committee composed of Dr. George Dock, Dr. W. S. Thayer, Dr. Joseph L. Miller, Dr. David Edsall, Dr. Theodore Janeway, and Dr. Richard Cabot to consider publication of a journal to present the more important researches of American students of clinical medicine. After the report of this committee was favorably acted upon the members were constituted as the first editorial board of the newly created ARCHIVES OF INTERNAL MEDICINE. As the Chicago member of the board, Dr. Miller was chosen as first editor-in-chief, with the stipulation that the other members were also to pass on the manuscripts submitted, a stipulation scrupulously carried out through the ARCHIVES' half-century of existence. There were a few changes in the board during the succeeding twenty-five years: Dr. Warfield T. Longcope succeeding Dr. Edsall in 1911, Dr. Hamman succeeding Dr. Janeway in 1913, and Dr. Walter W. Palmer replacing Dr. Hamman in 1924. In 1931, Dr. James H. Means succeeded Dr. George Dock. Dr. Miller continued as chief editor until 1932, when he was succeeded by Dr. N. C. Gilbert, and a new board composed of Dr. Arthur Bloomfield, Dr. Reginald Fitz, Dr. John H. Musser, and Dr. Russell M. Wilder was selected by the American Medical Association trustees. This distinguished group placed its stamp on the character of the ARCHIVES just as significantly as did the original board of 1907. Dr. Bloomfield now remains as the only active associate editor remaining from the 1932 group. All of the other present incumbents, whose terms go back no further than ten years, are deeply grateful for his wise counsel and steadying influence.

As a pioneer in the medical specialty field the ARCHIVES has been keenly aware of its obligation to carry on the high purposes of its founders as stated in the original announcement of its inauguration in *The Journal of the American Medical Association*: ". . . the Board of Trustees of the American Medical Association has taken steps for the founding of the ARCHIVES OF INTERNAL MEDICINE. It will be devoted to the publication of original studies on clinical medicine carried out at the bedside or in the laboratory, and of physiological, pathological and pharmacological researches that have a bearing on the nature, diagnosis or treatment of disease. Scholarly, thorough work, well abreast of the present position of medical science, is what is desired for the ARCHIVES OF INTERNAL MEDICINE. All technical details and protocols necessary to prove the thesis will be welcome, although unnecessary details are to be avoided. Articles that summarize what is already known on a certain topic and then add some-

thing new, represent the class of communications that are desired, as well as short original communications on matters relating to internal medicine."

The present ARCHIVES Editorial Board feels, as does its predecessors, that top priority should be given papers which present new facts or new concepts adequately supported by reliable clinical and laboratory observations. In addition, it plans to continue reviews of the new developments in various aspects of internal medicine by men qualified to select and comment upon the best in recent publications on the subject concerned. The book section has taken on new life under the able guidance of William Bean. Honest and penetrating evaluations of the amazingly numerous new volumes of special interest to internists are available every month in the pages of the ARCHIVES. In the new series which began last August under the aegis of Arthur Bloomfield—Evolution of Modern Medicine—various authorities have reviewed the steps by which modern understanding of various disease states came into being. In these scholarly treatises, some order has been restored to medical thinking.

The new department, which begins in the present issue under the somewhat formidable title of Contributions of Basic Science to Modern Medicine, will contain statements of fundamental concepts, painstakingly arrived at in the laboratory, which form the basis for the clinical application of new forms of diagnosis and therapy. For the hurried practicing internist, anxious to understand as well as to use new therapeutic tools intelligently, this series should be a godsend. It will prove equally useful to young men preparing for board examination and the exacting clinical and research tasks ahead of them. Dr. Charles Davidson has assumed special responsibility for this department. Realizing that its facets are many, he will welcome presentations from any of the preclinical disciplines which form the basis for useful clinical procedures. The paper by Dr. Richard W. Vilter and his associates, "Vitamin B_6 in Internal Medicine," is an excellent example of the kind of material that will make up the series.

With the present issue, Dr. George Burch takes over special supervision of editorials. No one is better able to survey the American medical scene or to choose able colleagues to comment upon it than this peripatetic member of our board. It is our confident belief that new life will be instilled into this important section of the ARCHIVES as has been done in the book section. The series on treatment of the varied conditions that confront the internist has been cordially received by our readers. It will continue under the special editorship of Dr. Hugh Butt.

As the eldest member of the family of specialty journals of the American Medical Association, the A. M. A. ARCHIVES OF INTERNAL MEDICINE belongs to the internists and medical investigators and teachers of this country. It is hoped they will approve of the new developments outlined above and participate actively in them.

PAUL S. RHOADS, M.D.,
Chief Editor.

Ulcerative Colitis—A Challenge

Ulcerative colitis is one of the least known yet most challenging problems of modern medicine. Though much less common than cancer, heart disease, or peptic ulcer, ulcerative colitis, nevertheless, is more frequent than is generally appreciated. It has been suggested also that the disease is increasing in frequency. Though differences in incidence are thought to exist between countries and continents, the geographic distribution of ulcerative colitis requires further study. A curious and unexplained feature is the apparent infrequency of ulcerative colitis in city or country hospitals contrasted with private institutions. Ulcerative colitis is not epidemic, infective, or transmissible. There is no satisfactory evidence of inherited susceptibility to the disease. Its presence in more than one member of a family is uncommon, though it occurs. Young people between the ages of twenty and forty and children are affected most often, but the disease may develop at all ages. It is commoner in females, but the difference in sex incidence is not great. Despite its severity, most patients survive the illness, though they may experience a life of discouraging invalidism. The economic burdens imposed by the frequent, prolonged, and expensive hospitalizations are staggering. The inability to establish a useful life creates serious social problems. On the other hand, the outlook is not uniformly gloomy; numerous patients improve, for one reason or another, indicating that the disease can be controlled—perhaps cured.

What then is ulcerative colitis? Its description simply as an acute or chronic inflammatory and ulcerative disease of the rectum and colon does not differ significantly from the original report of Wilkes and Moxon, in 1875. The illness is characterized clinically by rectal bleeding, diarrhea, abdominal pain, fever, anorexia, and weight loss and proctoscopically by diffuse inflammation, with hyperemia, edema, ulceration, bleeding, and sanguinopurulent exudation. The roentgenologic features of the disease are loss of the normal haustrations of the colon, narrowing, shortening, ulceration, and pseudopolypoid changes in the mucosa. These involve the entire large bowel in approximately fifty per cent of cases and varying portions in forty per cent; in perhaps ten per cent there are no obvious x-ray changes, presumably because the process is limited to the mucosa. The terminal ileum is affected in fifteen to twenty per cent of cases. Ileocolitis affects chiefly the distal portion of ileum, the cecum, and the ascending colon.

The course of ulcerative colitis is characterized by unpredictable recurrences and remissions, making accurate evaluation of therapy most difficult. The exacerbations are associated usually with emotional disturbances, upper respiratory infections and other illnesses, physical fatigue, dietary indiscretions, the use of antibiotics and cathartics, operations, and, in women, with the menses. The complications are numerous, including hemorrhage, perforation, peritonitis, stricture, obstruction, intestinal malabsorption, and polyps. The incidence of carcinoma of the colon and rectum in patients with ulcerative colitis is significantly higher than in the general population. Ulcerative colitis thus offers a unique opportunity to study the processes of inflammation, ulceration, and healing, with regenerative

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hyperplasia and the possible transition to neoplasia. The systemic complications include anemia; hypoproteinemia; rheumatoid arthritis; erythema nodosum; pyoderma gangrenosum; iritis; vascular thromboses; fatty liver; hepatitis and cirrhosis of the liver; lesions of the pancreas, kidneys, and adrenal glands, and depletion of electrolytes. There is no specific therapy. Medical management is symptomatic, individualized, and prolonged. The immediate objectives are physical and emotional rest, maintenance of nutrition, prevention or elimination of infection, and decreased activity of the colon. Treatment thus involves hospitalization; the use of sedatives, tranquillizing drugs, and antispasmodics; a nonirritating diet; sulfonamides; occasionally penicillin and streptomycin; blood transfusions; plasma; vitamins; iron; electrolytes; fluids, and intelligent supportive psychotherapy. In patients not responding to these measures, and especially in acute fulminating colitis, corticotropin and adrenal steroids may be useful adjuncts. Though not curative, they reduce the vascular and tissue response to inflammation and suppress the toxic reaction. Infinite patience and perseverance are necessary in the management of ulcerative colitis. Under such circumstances, medical treatment seems helpful in eighty to eighty-five per cent of the patients. In my experience, operation becomes necessary in perhaps ten to fifteen per cent of cases; in other series the frequency of surgery is higher. The procedure of choice is total colectomy, including the rectum, and ileostomy. While many persons are restored thereby to a useful life, surgery does not always solve the problem. Repeated hospitalization may be necessary subsequently because of complications involving the ileostomy, including bleeding, prolapse, retraction, stenosis, obstruction, or perforation. Extensive inflammation of the small intestine may ensue, creating an extremely serious situation. Despite the lack of specific treatment, the mortality rate in ulcerative colitis during the past fifty years has decreased from fifty to approximately five to ten per cent. Occasionally there is striking improvement, with reversibility of the diseased bowel toward normal.

The etiology and pathogenesis of ulcerative colitis remain obscure. The inflammation noted at proctoscopy, the febrile course, the complications of abscess and fistula, and the response to sulfonamides suggest an infection. Innumerable bacterial agents have been implicated, but none have been established as causes. Ulcerative colitis develops occasionally after recovery from amebic dysentery, the sequence suggesting a direct but obscure relationship, perhaps tissue sensitization. Of interest also is the occasional history of an acute enteric infection affecting several people but severer and more prolonged in the patient subsequently developing colitis. Viruses have been implicated theoretically because of their apparent relationship to certain enteric infections; investigation of this possibility has been undertaken only recently. Despite the many negative studies, microorganisms as direct or indirect factors in the evolution of ulcerative colitis have not been excluded completely. Further attention might be directed to the relatively unexplored anaerobic flora of the intestine; the potential pathogenicity of the "nonpathogenic" bacteria in the bowel, including coliforms, and perhaps to the elaboration of endotoxins, proteolytic enzymes, and other chemical substances from bacterial cells or as a consequence of bacterial action upon the mucosa of the rectum and colon.

An important relationship exists between emotional problems and ulcerative colitis. Physiologically, nervous tension may produce hyperfunction of the colon,

hyperemia, hemorrhage, and excessive secretion of mucus. An interesting experimental observation is the apparent development of an ulcerative colitis, as well as duodenal ulceration, in monkeys with electrodes implanted intracerebrally, observed under conditions permitting and producing anxiety and indecision concering intracerebral self-stimulation; monkeys subjected only to restraint for similar periods did not develop gastrointestinal lesions. Anatomically the number of autonomic nerve ganglia in the bowel wall apparently is increased in patients with ulcerative colitis, suggesting a neurophysiologic mechanism for the psychogenic stimuli. Clinically, the onset and the recurrence of the disease often are associated with emotional disturbances. The incidence of emotional disorders in patients with ulcerative colitis is very high. Characteristic personality traits have been suggested, including immaturity, insecurity, dependence, narcissism, "hypersensitivity," excessive precision and neatness, indecision, and inner hostility but outward passiveness. It is of interest that patients with steatorrhea have been described similarly. Intelligent psychotherapy may, indeed, facilitate striking clinical improvement, at times excelling the results of medical management. The response, however, may reflect chiefly the control of physiologic concomitants of the disease, rather than the fundamental cause. Many problems in this area require further investigation, including the specificity of the psychiatric features, the emotional disturbances encountered in children with ulcerative colitis, the selection of the colon as the end-organ for the psychogenic influences, and the influence of the disease and its control upon the personality of the patient. The importance of adequate controls and objective criteria has been emphasized previously. It seems evident that descriptions of personality traits and behavioral patterns, and conventional physiologic measurements, while of interest, will not clarify the nature of the disease. Perhaps attention now should be directed to the biochemical mechanisms involved in the tissue response to neurogenic and psychogenic influences, possibly the elaboration of acetylcholine, epinephrine, and other as yet unidentified substances, eliciting the tissue release of histamine, serotonin, "capillary permeability factors," and other agents, perhaps from mast cells.

The localization of the pathologic changes primarily in the mucosa and submucosa has suggested the role of proteolytic substances acting upon the surface of the bowel. While lysozyme no longer is regarded as an etiologic factor, the possible release of other substances from bacteria, or tissue cells, might be investigated. The nature of the tissue reaction in ulcerative colitis also remains to be elucidated. The serum mucoprotein values are elevated, paralleling the trends observed in rheumatoid arthritis and "collagen" diseases; however, similar increases are noted in patients with neoplasms and chronic infectious diseases. The increases in serum lactic dehydrogenase and pyruvic glutamic transaminase observed recently in occasional persons with ulcerative colitis probably are nonspecific reflections of tissue injury. On the other hand, further study may identify other enzyme systems or chemical substances more closely identified with the colon and disease.

The frequency of ulcerative colitis among young people is of interest perhaps in relation to the concept of "tissue hyperreactivity." Occasionally there is a striking multiplicity of diseases in the same person, as in a young woman with scleroderma, rheumatoid arthritis, febrile episodes resembling lupus erythematosus, and ulcerative colitis or in a young man developing successively rheumatic

fever and rheumatic heart disease, recurrent streptococcic pharyngitis, pneumonitis, ulcerative colitis, hepatitis, hyperglobulinemia, and fatal hemolytic anemia. The possible participation of a hypersensitivity mechanism, perhaps an autoimmune reaction in the colon, in the evolution of the disease has interested me for many years. The association of ulcerative colitis with rheumatoid arthritis, rheumatic fever, erythema nodosum, iritis, lupus erythematosus, scleroderma, and periarteritis nodosa, though not common, nevertheless is intriguing in this connection. Immunological disturbances, including reactions to drugs and blood transfusions, hemolytic anemia, and alterations in the serum proteins, chiefly increases in the α_2- and γ-globulins, occur in ulcerative colitis. The favorable clinical and tissue responses to corticotropin and adrenal steroids would not be incompatible with this concept. It is of interest, therefore, that the colon of the rabbit is capable of responding to the Arthus test and to the Shwartzman phenomenon, as has been demonstrated repeatedly in our laboratory. Of interest also in our laboratory has been the production of a "colitis" in rabbits sensitized to egg albumen, with use of techniques presumably promoting the localization of antigen and antibody in the colon. This intriguing aspect of the problem obviously requires further study. There have been relatively few attempts to reproduce the disease experimentally. "Colitis" apparently has been induced in animals by deficiencies of vitamin A and folic acid, mesenteric lymphatic obstruction, administration of histamine, and the injection of cholinergic drugs, such as carbachol (Doryl), methacholine (Mecholyl), and neostigmine (Prostigmin). Continued injection of parasympathomimetic compounds produces a hyperemic friable rectal mucosa, not unlike the appearance of mild ulcerative colitis in man; however, the bowel returns to normal rapidly when the injections are discontinued. Parasympathomimetic stimulation of the colon, in the presence of diminished tissue cholinesterase, may induce more intense and sustained muscular contraction of the colon, with hemorrhage of the mucosa and submucosa. Recognizing the limitations of species differences, further experimental studies would appear worth while, for the identification and clarification of any single potential factor would contribute to the better understanding of the disease. Animal studies probably would be of interest in studying the tissue reaction and the associated biochemical responses of the colon to experimentally induced inflammation and to chemical or immunologic injury.

The anatomic changes vary with the stage and severity of the disease and with the presence or absence of complications. The acute phase is characterized by vascular congestion, edema, hemorrhage, and ulcerations; the mucosa is infiltrated with many lymphocytes, plasma cells, and eosinophils; variable numbers of polymorphonuclear cells are present in the lamina propria and in small crypt abscesses; the number of goblet cells is reduced; the crypts are diminished and are arranged abnormally. Vasculitis and crypt abscesses are observed occasionally.

In chronic ulcerative colitis the bowel wall is thickened and fibrotic. The histologic features include vascular engorgement, cell infiltration, fibrosis, fatty infiltration of the submucosa, hypertrophied muscle layers, prominent autonomic nerve ganglia, and pseudopolyps. In approximately thirty per cent of cases, in our experience, granulomata with or without giant cells are observed. Study of the anatomic features of ulcerative colitis during the initial phase of the disease could be important, for present information is based usually upon the examination

of colons subjected to multiple episodes of colitis and to secondary infection, possibly obscuring the primary tissue response.

Additional theories as to the cause of ulcerative colitis include nutritional deficiency, allergy to foods or to pollens, lymphatic obstruction, the lack of protective agents in the bowel wall, alterations in the ground substance of the connective tissue of the colon, vasospastic phenomena induced by parasympathetic stimulation, and metabolic disorders. The etiologic possibilities thus encompass many scientific disciplines. This devastating and intriguing disease deserves more attention than it has received heretofore. Careful clinical observation and standard laboratory procedures will remain useful guides to the course and management of the disease. However, solution of the problem of ulcerative colitis obviously requires more than conventional techniques. There is great need for new ideas; for the enlistment of basic sciences, perhaps biochemistry and immunology in addition to pathology and physiology, in future research, and for well-trained imaginative investigators, interested in the problem and unhampered by tradition. Attention might be directed to the biochemistry and enzymology of inflammation, injury, and hypersensitivity of the colon; the nature of the healing process in the colon; vascular disturbances; elaboration of bacterial endotoxins; the liberation of acetylcholine and other substances; the role of histamine and serotonin; the identification of other chemical and humoral agents, and perhaps to the biologic behavior of the colonic mucosa and submucosa as observed in tissue-culture studies. Does ulcerative colitis occur in particular persons, under specific circumstances, or does it reflect a nonspecific response to multiple causes? What are the factors contributing to the susceptibility to the disease? Is there a pathogenetic relationship between regional enteritis and ulcerative colitis? Many other problems undoubtedly will suggest themselves for further investigation. The challenge of ulcerative colitis extends far beyond the colon. In certain respects it encompasses much of medicine and science.

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A.M.A. ARCHIVES OF
INTERNAL MEDICINE

An "Autoimmune" Reaction Against Human Tissue Antigens in Certain Acute and Chronic Diseases

I. Serological Investigations

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With the Technical Assistance of Lois Larkin

During the course of investigations aimed at detecting infectious hepatitis virus antigen in human livers from patients dying early in the course of fulminating infectious hepatitis with acute yellow atrophy of the liver, many human sera were found to react with liver antigen with fixation of complement in a manner indistinguishable from classical antigen-antibody complement-fixation reactions. It has subsequently been possible to demonstrate specific complement fixation, in some cases in extraordinarily high titer, with human sera from patients suffering from a number of different diseases, using saline suspensions of finely homogenized normal human tissues as antigens. In a preliminary communication [1] I reported study of 190 human sera by this technique, with the finding of high incidence of positive reactions in disseminated lupus erythematosus, "lupoid hepatitis" (see below), other chronic hepatitides, and macroglobulinemia. The current report is concerned with extension of these studies in these and other diseases and to animals immunized with tissue antigens. Details of the technique employed are presented, and further characterization of both the antigens and the serum reagents, presumably auto-antibodies, is reported. More extensive correlation of this autoimmune complement-fixation (AICF) reaction with the clinical course and other laboratory findings is presented in the second paper of this series.[2]

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Materials and Methods

Antigen Preparation.—Normal human tissue, removed less than 12 hours post mortem, was washed free of surface blood in isotonic saline solution, finely minced with sharp scissors in a Petri dish, and ground in a chilled mortar with sterile sand, with use of isotonic saline solution chilled to 4 C as diluent. The larger tissue debris was removed from the 10% suspension by centrifugation at 500 g for 15 minutes. The supernatant was respun at 1000 g for 15 minutes and the sediment again discarded. After each centrifugation low-density fatty material, which formed a top layer in homogenates of certain tissues, was discarded. The second supernatant was then centrifuged at 2000 g for 15 minutes, and the supernatant from this third spinning was stored at —70 C in glass-sealed ampules. The final supernatants served as crude tissue antigens for use in the complement-fixation test and as starting material for further antigen fractionation. Such antigens were prepared from liver, kidney, skeletal muscle, cardiac muscle, spleen, pancreas, thyroid, adrenal, prostate, lung, stomach, skin, peritoneum, aorta, and pulmonary artery. Similar antigens were prepared from normal human embryo liver, kidney, heart, skin-muscle, and brain. In a few instances grossly pathological human tissues were used for the preparation of special antigens. Thus, antigens were

made from liver in cases of acute yellow atrophy viral hepatitis, Hodgkins-disease, and severe biliary cirrhosis.

Identical procedures were used for the preparation of antigens from tissues of the rat, rabbit, mouse, and other species.

In later investigations, when large batches of antigen were being prepared, the scissor mincing was replaced by grinding in a Waring Blendor at 4 C for three minutes. Antigens were then stored at —20 C instead of —70 C, and no appreciable loss of activity was observed during one month of storage.

Sera.—Sera, separated centrifugally from clotted blood specimens collected under sterile conditions, were stored at —20 C within 24 hours of collection. To avoid repeated freezing and thawing small aliquots were often placed in separate tubes.

All sera, unless otherwise specified, were inactivated at 56 C for 30 minutes at a 1:4 dilution in calcium magnesium saline * immediately before titration. Occasionally sera were inactivated undiluted or, if of high titer, were diluted 1:16.

Complement-Fixation Test.—The complement-fixation (CF) method used was based essentially on that of Donnelley,[3] two 50% hemolytic units of complement being used and fixation carried out at 37 C for 30 minutes. Complement was always titrated in the presence of antigen. The chief modification was the use of Perspex trays with multiple cups instead of the conventional tubes. A full range of serum and antigen controls was included in all tests. Titers were read as the reciprocal of the highest dilution giving 2+ fixation or greater. Standard dropping pipets delivering 25 drops per milliliter of calcium-magnesium saline solution at room temperature were used in preparing this 4 drop CF reaction. All titers of 64 or higher were checked using serial two-fold dilutions made by changing pipets for each successive dilution. Sera reacting at dilutions over 1:1000 were retitrated by starting with an initial 1:1000 dilution prepared by the use of an accurate volumetric pipet and volumetric flask.

Antigen Titrations.—Block titrations were made of all antigens, with use of a known-positive human serum. Titer decreased slowly with increasing anti-

* Composition: NaCl 8.50 gm., $CaCl_2$ (anhydrous) 0.0275 gm., $MgCl_2$ (crystalline) 0.192 gm., H_3BO_3 (crystalline) 1.203 gm., $Na_2B_4O_7 \cdot 10\ H_2O$ (crystalline) 0.052 gm. Dissolved and made up to 1 liter of distilled water.

TABLE 1.—*Complement-Fixation Reactions of Human Sera with Human Liver and Kidney Antigens*

Clinical Condition	Subjects, No.	Liver Antigen				Kidney Antigen			
		Titer			% Positive	Titer			% Positive
		Neg.	4–16	>32		Neg.	4–16	>32	
Normal	187	183	4		2	165	20	2	12
Miscellaneous diseases	152	147	5		3	141	11		7
Hepatic diseases									
Viral hepatitis									
(a) Acute									
1st wk.	10	7	3		30	6	3	1	40
2d wk.	25	21	4		16	13	12		48
3d wk.	15	10	5		33	5	9	1	67
4th wk.	11	10	1		9	7	4		36
2d mo.	16	13	3		19	7	9		56
3d mo.	6	6			0	4	2		33
Over 3 mo.	8	8			0	8			0
(b) Chronic active hepatitis	9	2	7		78		9		100
(c) Lupoid hepatitis	4	1	1	2	75	1	2	1	75
Chronic nutritional hepatitis	11	8	3		27	8	3		27
Chronic biliary cirrhosis									
(a) Primary (nonobstructive)	1			1	25			1	50
(b) Obstructive	7	6	1			4	3		
Collagen diseases									
Acute rheumatic fever	5	4	1		20	3			0
Rheumatoid arthritis	6	6			0	3	3		50
Acute glomerulonephritis	5	3	2		40	3	2		40
Nephrotic nephritis	3	2	1		33	3			0
Disseminated lupus erythematosus	12	3	7	2	75	2	8	2	83
Paraproteinemias									
Multiple myeloma	3	2	1		33	2	1		33
Macroglobulinemia	5	3		2	40	2	1	2	60
Other diseases									
Syphilis									
Primary	1		1				1		
Tabes dorsalis	1	1				1			
Infectious mononucleosis	2	1	1			1	1		
Sarcoid	3	3				2	1		
Hemochromatosis	1		1				1		
Actinomycosis (involving the liver)	1		1				1		
Ulcerative colitis	2	2				1	1		

gen dilution, and high titer sera gave a distinct prozone with diluted antigens. Therefore, the 10% tissue antigens were used undiluted, or diluted to 5% (1:2) for those few antigens which consumed over one 50% hemolytic unit of complement at full strength (10%). At this dilution none of the antigens consumed over 1 unit of complement.

Results

Table 1 summarizes the results of CF reactions with use of human liver and kidney antigens on sera from 496 subjects. To serve as controls, sera from 187 normal persons and 152 persons suffering from a variety of acute and chronic diseases, grouped in the Table as "miscellaneous diseases," were examined. Four normal sera (2%) and five from diseased persons (3%) reacted with liver antigen at low titers (i. e., 16 or lower). The four reacting normal sera all had titers of 4 only. The five positive pathological sera were all from patients with undiagnosed diseases whose syphilitic serology was negative; one had a titer of 4, two had a titer of 8, and two had a titer of 16. Twenty-two normal sera (12%) and eleven pathologic sera (7%) reacted with kidney antigen. The four normal sera which had reacted with liver at titers of 4 reacted with kidney at higher titers. The 22 reacting normal sera gave the following titer distribution:

Titer	Sera, No.
4	13
8	6
16	1
32	2

Seven of the 11 kidney-reacting pathologic sera were from patients with undiagnosed diseases whose syphilitic serology was negative. Two of these also reacted with liver antigen. The four other reactive sera were from subjects suffering from the following diseases: acute viral influenza, one; chronic pancreatitis, one, and metastatic gastric carcinoma, two. None of these pathologic sera had titers over 16. Two specimens from the patient suffering from acute viral influenza—one collected early in the acute phase and one during convalescence, two weeks later—both gave the same titer, of 4, with kidney antigen and no reaction with liver. Three separate specimens from the patient with gastric carcinoma with liver metastases collected at weekly intervals were all positive at titers of 4 or 8.

The 152 pathologic specimens were obtained from patients with the following diseases: acute upper respiratory infections including the common cold and viral influenza, 28; viral pneumonia, 1; bronchopneumonia, 1; bronchiectasis, 2; chronic bronchitis with emphysema, 1; malignant neoplasms, 11 (including chronic lymphatic leukemia, 1; chronic myelogenous leukemia, 1; lymphosarcoma, 2, and metastatic carcinoma, 7); eosinophilic granuloma, 1; aplastic anemia, 2; pernicious anemia, 1; thrombocytopenic purpura, 1; chronic pancreatitis, 11; chronic gastritis, 3; pyelonephritis, 3; Paget's disease, 2; serum sickness, 1; duodenal ulcer, 3; gastric ulcer, 1; myxedema, 1; myocardial infarct, 1; cerebrovascular accident, 1; familial xanthomatosis, 1; essential hypertension, 2; thyroiditis, 2 (including Hashimoto's post-thyroidectomy, 1, and recovered acute thyroiditis, 1); mumps, 1; gout, 2; pyogenic arthritis, 2; tubercular arthritis, 2; burns, 2; compound fractures, 5; undiagnosed diseases, 9, and diverse diseases, serum from which was sent to the laboratory for syphilitic serology or hydatid CF reactions, 48.

The remainder of Table 1 summarizes results of liver and kidney antigen CF reactions in those diseases in which either the incidence or the magnitude of reaction significantly exceeds that found in normal subjects or in the group of miscellaneous diseases. These conditions may be conveniently grouped into four divisions: hepatic diseases, collagen diseases, paraproteinemias,[4] and a final group of a few cases of other diverse systemic diseases, including syphilis, infectious mononucleosis, sarcoid, actinomycosis (involving the liver), hemochromatosis, and ulcerative colitis.

It will be noted that high-titer (i. e., above 16) reactions—presumably reflecting autoantibody levels—were found only in a

few pathological conditions, namely, in disseminated lupus erythematosus, acute viral hepatitis, "lupoid hepatitis," primary biliary cirrhosis, and macroglobulinemia. In each of these conditions some sera with titers of 64 or higher were encountered, and in the last three diseases sera with titers of over 1000 were found.

Although all preliminary work was done with use of liver and kidney antigens, extracts prepared from skeletal muscle, cardiac muscle, spleen, pancreas, thyroid, and adrenal were subsequently used. Sera negative with liver and kidney antigens generally remained negative with these other tissues. On the other hand, sera positive to liver and kidney usually reacted also with all other antigens except, rather frequently, with pancreas. This latter antigen reacted with fewer sera than did other antigens. Its low activity may be the result of enzymatic action during antigen preparation.

Sera positive with human liver, kidney, and muscle antigens were titrated with use of other human organ antigens and a number of antigens prepared from human embryos and from other species. Representative results are summarized in Tables 2 to 10, inclusive, while correlation of these autoimmune reactions with the clinical course and laboratory data on individual patients is presented in the following paper.[2]

Hepatic Disease.—Viral Hepatitis: In Table 1 the results of the AICF test on 91 serum specimens from 75 patients are tabulated according to the time since onset of symptoms. It is evident that during the first two months of the disease, starting already in the first week, the incidence of positive reactions to liver antigens greatly exceeds that found either in normal subjects or in the group of illnesses collected together under the designation "miscellaneous diseases." During convalescence, after the second month from the onset of illness, these reactions are no longer encountered in cases progressing to recovery. With kidney antigen the same picture is found, although

TABLE 2.—*Acute Infectious Hepatitis*

Case	Time Since Onset, Days	Titer with Human Antigens *							
		L	K	M	S	H	P	T	A
HA1	8	±	±	128	±	±	0	0	0
	21	0	4	32		0		±	0
	34	0	4	0	0	±	0	0	0
HA2	9	4	8	4	4	0	0	4	0
	17	4	4	0	0	0	0	±	0
HA3	9	0	8	0	8	±	0	0	0
	29	0	0	±	0	±	0	±	0
HA4	11	0	4	0	4	0	±	0	0
	17	0	0	0	±	0	0	0	0
HA5	8–14	8	4	±	±	±	0	0	0
	15–21	4	8	8	32	4	0	8	0
	c. 75	0	4	±	0	0	±	±	0
HA6	8–14	0	16	±	8	4	0	8	0
	15–21	4	8	4	4	4	±	0	0
HA7	8–14	0	8	8	4	±	4	±	0
	c. 100	0	0	0	0	0	4	0	0
HA8	8–14	±	4	8	4	±	0	0	0
	c. 35	0	4	8	4	0	0	±	0
	c. 56	0	±	0	0	0	0	0	0
HA9	14	±	8	128	64	4	0	±	0
	21	8	32	>128	8	±	0	±	0
	30	0	±	±	0	0	0	4	0
HA10	35	0	4	8	±	4	0	±	0
	50	4	4	±	4	0	0	0	0
HA11	39	4	4	0	±	0	0	0	0
	53	±	8	0	4	4	0	±	0
	70	±	±						
	90	±	±	0	0	4	0	0	0

* The following abbreviations are used in Tables 2-10, inclusive: L, liver; K, kidney; M, skeletal muscle; S, spleen; H, cardiac muscle; P, pancreas; T, thyroid; A, adrenal.

TABLE 3.—*Chronic Postviral Hepatitis*

Case	Date	Titer with Human Antigens *							
		L	K	M	S	H	P	T	A
		Chronic Active Hepatitis							
HC1	10/9/56	8	8	4	4	0	0	4	±
	11/26/56	0	4	16	0	4	0	4	4
	12/3/56	0	8	0	±	0	0	±	4
	12/11/56	0	16	0	4	0	0	4	±
	12/19/56	0	0	±	0	0	0	0	0
	1/9/57	8	±	4	0	4	0	64	8
HC2	5/25/56	4	8	8	4	4	0	4	0
	7/20/56	4	8	4	4	4	0	4	0
	10/24/56	4	4	8	4	±	0	4	0
	12/19/56	0	0	0	0	0	0	0	0
HC3	9/17/56	8	16	16	±	0	±	4	0
HC4	9/12/56	4	±	4	4	4	0	±	0
	12/12/56	0	4	0	4	4	0	0	0
	1/3/57	±	4	0	±	0	0	0	0
HC5	5/23/56	±	0						
	7/4/56	4	4	0	0	0	0	4	0
	11/5/56	±	4	4	0	0	0	±	0
	12/12/56	0	0	0	0	0	0	0	0
	1/9/57	0	±	±	0	0	0	0	±
HC6	7/4/56	±	±	4	±	±	0	4	0
	9/13/56	4	4	±	±	0	0	0	0
	12/17/56	0	0	0	0	0	0	0	0
HC7	10/19/56	±	4	16	0	0	0	8	0
HC8	7/25/56	±	4	0	8	0	0	0	0
	10/19/56	4	±	4	4	0	0	±	0
	1/24/57	0	0	0	0	0	0	0	0
HC9	12/18/56	0	4	4	±	4	0	4	4
		Lupoid Hepatitis							
HL1	5/25/56	2,048	>2,048	512	64	32	8	1,024	2,048
	6/6/56	>2,048	>2,048	256	32	16	±	1,024	512
	9/20/56	512	2,048	512	±	8	0	1,024	512
	11/13/56	1,024	2,048	1,024	0	1,024	0	512	4,096
	1/9/57	512	2,048	512	0	4	0	2,048	512
HL2	8/-/56	64	16	8	32	8	0	8	16
HL3	1/4/57	32	8	8	0	16	0	16	8
HL4	6/18/56	±	8	4	0	0	0	8	0
	7/6/56	4	16	4	4	0	±	4	0
	9/19/56	±	16	0	0	0	0	4	0
	10/9/56	±	4	±	0	0	0	4	0
	12/12/56	0	0	±	0	0	0	0	0
HL5	10/23/56	4	8	0	8	0	±	±	16
	11/5/56	0	0	±	4	0	0	±	±
	12/12/56	0	4	0	±	0	0	4	8

* See Table 2 for key to abbreviations.

TABLE 4.—*Chronic Nutritional Hepatitis*

Case	Date	Titer with Human Antigens *							
		L	K	M	S	H	P	T	A
HN1	9/7/56	8	4	4	0	4	0	8	8
HN2	9/17/56	16	0	8	4	±	4	32	16
HN3	11/5/56	0	4	4	0	4	0	8	8
	11/12/56	0	4	4	0	4	0	8	4
	12/7/56	±	4	4	0	±	±	4	4
	12/19/56	4	4	4	4	±	0	4	0
	11/3/57	4	4	±	4	0	0	4	±
HN4	12/14/56	4	4	4	±	±	0	4	4
HN5	10/9/56	4	4	4	±	4	0	8	8
HN6	8/-/56	±	4	4		±	4	8	4
HN7	11/2/56	±	0	4	0	±	0	4	±
HN8	8/21/56	0	4	4	±	±	0	16	4
	11/9/56	4	4	8	4	4	8	8	8
HN9	11/16/56	±	0	4	0	4	0	8	8
HN10	11/12/56	4	4	8	0	4	0	8	8
HN11	2/27/56	±	0	4	0	4	0	8	8

* See Table 2 for key to abbreviations.

TABLE 5.—*Chronic Biliary Cirrhosis*

Case	Date	Titer with Human Antigens *							
		L	K	M	S	H	P	T	A
				Primary					
HB 1	7/28/56	64	128	64	0	128	0	128	128
	8/8/56	1,000	4,000	16	8	128	0	32	256
	8/15/56	1,000	4,000	±	±	64	0	128	512
	8/22/56	2,000	4,000	8	16	128	0	64	512
	9/12/56	512	1,000	32	4	32	16	64	256
	9/21/56	2,000	2,000	32	0	64	0	32	1,000
	11/14/56	1,000	4,000	128	8	256	8	128	512
	12/12/56	2,000	2,000	512	8	128	0	2,000	2,000
	1/4/57	4,000	4,000	64	4	128	0	1,000	256
				Obstructive					
HB1	8/-/56	4	4	4	4	0	0	4	4
	11/2/56	8	4	±	4	0	0	0	±
	12/7/56	4	8	4	0	0	0	±	0
HB2	10/-/56	0	0	4	0	0	0	±	±
	11/26/56	0	4	0	0	±	0	0	0
	12/3/56	±	4	±	0	±	0	0	0
	12/17/56	0	0	4	0	0	0	0	0
HB3	11/14/56	4	16	±	8	0	0	16	8
	12/12/56	4	16	4	16	0	0	8	8
HB4	11/12/56	0	4	±	0	0	0	0	±
	12/8/56	0	0	0	0	0	0	0	0
HB5	8/4/56	0	0	0	0	0	0	0	0
HB6	10/24/56	0	0	0	0	0	0	0	0
HB7	2/27/56	0	0	0	0	0	0	0	0

* See Table 2 for key to abbreviations.

positive reactions are still frequent during the third month from the onset of illness.

Acute infectious (viral) hepatitis. Table 2 presents the results of 11 representative cases of acute viral hepatitis studied with a number of different antigens. Since too few specimens were obtained very early in the course of the disease and none in the incubation period, no paired sera were available with which the development of the active reagent could be observed. In a number of cases, however, the disappearance of positive reactions upon recovery was noted (HA3, HA4, HA7, HA8, HA9, HA11).

Certain sera reacted more strongly with muscle antigens than with liver or kidney antigens (HA1, HA8, HA9, HA10). Although titers have not been as reproducible with various batches of skeletal muscle antigen as with different liver and kidney antigens, it was found advisable to screen acute viral hepatitis sera for positive reactions with use of all three antigens.

Chronic active (postviral) hepatitis. Although no high-titer sera were encountered in the nine patients studied with this condition, a high percentage of these patients showed low-titer reactions with liver (78%) and kidney (100%) antigens. The results of study of 26 sera from these nine patients are presented in Table 3. It is evident that certain sera gave stronger reactions with muscle antigen than with either kidney or liver, as was the case in acute viral hepatitis. Thus, in this condition it was again advisable to screen sera with liver, kidney, and muscle antigens. It proved more difficult to prepare skeletal-muscle antigens which gave consistently reproducible reactions than liver or kidney antigens. Thus, although all muscle-antigen preparations have been active, serum titers with various muscle antigens have varied greatly, whereas this has not been the case with other antigens.

Lupoid hepatitis. This progressive disseminated lupus erythematosus-like syndrome supervening in the course of chronic active (viral) hepatitis has recently been described by Mackay et al.[5] The lower portion of Table 3 summarizes the reactions of 15 sera from these five patients to eight different human tissue antigens. As with chronic active hepatitis, this syndrome gave frequent positive reactions with liver

TABLE 6.—*Collagen Diseases*

Case	Date	Titer with Human Antigens *							
		L	K	M	S	H	P	T	A
		Acute Rheumatic Fever							
RF1	8/–/56	16	0	0	0	0	0	0	0
	11/2/56	4	0	0	0	0	0	0	0
RF2	10/9/56	±	±	0	0	0	0	0	0
	12/19/56	0	0	0	0	0	0	0	0
RF3	11/16/56	0	±	0	0	0	0	0	0
RF4	1/16/57	0	0	0	0	0	0	0	0
	2/1/57	0	0	0	0	0	0	0	0
RF5	1/21/57	0	0	0	0	0	0	0	0
	2/1/57	0	0	0	0	0	0	0	0
		Rheumatoid Arthritis							
RA1	8/–/56	0	4	4	16	4	0	4	0
	9/–/56	0	8	8	8	0	0	0	0
	12/15/56	0	0	0	±	0	0	0	0
RA2	11/21/56	0	4	4	0	0	0	0	0
	12/12/56	0	4	0	0	0	0	0	0
RA3	11/26/56	0	4	0	±	0	0	4	0
RA4	8/–/56	0	0	0	0	0	0	4	4
	11/16/56	0	0	0	0	0	0	0	0
RA5	11/28/56	0	0	0	0	0	0	0	0
	12/19/56	0	0	0	0	0	0	0	0
RA6	12/14/56	0	0	0	±	0	0	0	0
		Acute Glomerulonephritis							
NA1	11/23/56	0	±	8	0	4	0	0	0
	12/4/56	4	4	4	4	4	0	0	0
	12/10/56	0	4	0	0	0	0	0	0
	1/9/57	±	0	4	0	0	0	4	4
NA2	8/–/56	4	4	8	4	4	0	0	0
	10/12/56	0	4	4	4	±	0	±	4
	11/28/56	4	4	16	8	16	0	0	0
	1/4/57	4	0	4	0	4	0	8	±
NA3	12/12/56	0	0	0	0	0	0	0	0
NA4	8/–/56	0	0	0	0	0	±	0	±
	1/4/57	0	0	0	0	4	0	±	±
NA5	1/21/57	0	0	0	0	0	0	0	0
	2/1/57	0	0	0	0	0	0	0	0
		Nephrotic Nephritis							
NN1	11/–/56	±	±	4	0	4	0	4	8
NN2	8/30/56	0	0	0	0	0	0	0	0
	12/19/56	0	0	0	0	0	0	0	0
NN3	8/–/56	4	0						

* See Table 2 for key to abbreviations.

(100%) and kidney (100%) antigens. Two cases (HL2 and HL3) reacted with liver at titers of 64 and 32, while in another case (HL1) titers of over 1000 were found to both liver and kidney.

Chronic Nutritional Hepatitis: Table 4 summarizes the AICF reactions with eight human organ antigens of 16 sera from 11 adult patients with chronic nutritional hepatitis associated with the excessive consumption of alcohol. Seven of the eleven patients (64%) reacted with kidney antigen, and seven (64%), with liver antigen; at least one serum specimen of six of these patients (HN1, HN3, HN4, HN5, HN8, HN10) reacted with both antigens. Sera from all cases (100%) reacted with muscle and thyroid antigens and from 10 cases (91%), with adrenal antigen.

Diverse organ specificities of the serum reagents were encountered. Thus, Case HN2 reacted with liver but not with kidney, while, on the other hand, Case HN6 reacted with kidney but not with liver. Titers were low in all instances, the highest being a reaction of serum HN2 with liver and adrenal at a titer of 16 and with thyroid at a titer of 32 and of HN8 with thyroid at

TABLE 7.—*Disseminated Lupus Erythematosus*

Case	Date	Titer with Human Antigens *							
		L	K	M	S	H	P	T	A
L1	11/28/56	128	128	128	128	128	0	256	128
	12/7/56	256	256	128	128	128	0	256	64
L2	11/1/56	32	64	8	8	0	0	16	16
	11/26/56	4	8	8	4	0	0	4	4
L3	8/17/56	8	8	8	8	4	±	8	4
	9/14/56	8	8	8	8	±	4	8	4
	11/16/56	8	16	16	16	8	8	4	8
	2/1/57	16	16	4	16	4	4		
L4	9/12/56	4	8	4	4	4	0	4	4
	11/21/56	4	16	4	4	0	0	4	4
L5	9/20/56	4	8	4	8	4	0	4	8
	11/8/56	8	16	16	32	0	0	4	8
	12/7/56	4	4	0	8	0	0	4	0
L6	11/7/56	8	8	8	±	8	0	0	4
	11/20/56	4	4	8	±	0	0	0	±
	12/12/56	±	16	0	0	0	0	±	0
L7	9/15/56	8	8	8	8	8	0	±	8
	12/5/56	4	8	4	4	4	0	4	8
	1/9/57	4	0	0	0	0	0	0	4
L8	10/1/56	4	8	8	4	0	0	±	8
	10/9/56	4	8	8	4	±	0	4	8
	1/24/57	8	8	0	4	4	0	±	4
L9	8/20/56	8	8	±	0	±	0	±	4
L10	12/10/56	±	4	0	256	0	0	0	4
	12/13/56	±	4	±	256	0	0	0	4
L11	11/28/56	0	0	±	0	0	0	0	0
L12	11/28/56	0	0	0	0	0	0	0	0
	12/19/56	0	0	0	0	0	0	0	0

* See Table 2 for key to abbreviations.

a titer of 16. Of the eight antigens, skeletal muscle, thyroid, and adrenal would be as satisfactory as liver and kidney for screening sera for positive reactions. Two patients (HN3 and HN8) on whom serial specimens collected over the course of three months were available developed positive reactions to liver during the period of observation.

Chronic Biliary Cirrhosis: Table 5 summarizes the reactions of 9 sera from one patient with primary nonobstructive chronic

TABLE 8.—*Paraproteinemias*

Case	Date	Titer with Human Antigens *							
		L	K	M	S	H	P	T	A
		Multiple Myeloma							
MY1	8/-/56	4	4	4	0	4	0	4	4
MY2	10/-/56	0	0	0	0	0	0	0	0
MY3	11/9/56	0	0						
	12/21/56	0	0	0	0	0	0	0	0
MY4	1/9/57	0	0	0	0	0	0	0	0
		Macroglobulinemia							
MG1	8/22/56	16,000	2,000	128	8	16	16	64	500
	8/24/56	16,000	4,000	32	8	8	8	64	500
	8/29/56	8,000	2,000	2,000	8	8	16	256	1,000
	11/13/56	32,000	4,000	8,000	1,000	2,000	16	4,000	4,000
	12/13/56	32,000	8,000	8,000	8	8	8	4,000	2,000
MG2	10/-/56	512	1,000	2,000	64	1,000	0	128	256
	1/8/57	1,000	1,000	1,000	0	0	0	±	±
MG3	9/-/56	0	0	0	0	0	0	0	0
	12/12/56	0	8	±	0	0	0	±	0
MG4	11/-/56	0	0	0	0	0	0	0	0
MG5	11/-/56	±	0	0	0	±	0	0	0

* See Table 2 for key to abbreviations.

TABLE 9.—*Other Diseases Giving Positive Autoimmune Complement-Fixation (AICF) Reactions*

Case	Diagnosis	Titer with Human Antigens *							
		L	K	M	S	H	P	T	A
M1	Syphilis, primary	16	16	16	0	8	+	8	32
M2	Syphilis, tabes dorsalis	0	0	0				0	0
M3	Infectious mononucleosis	4	16	8	8	4	4	8	0
M4	Infectious mononucleosis	0	0	0	0	0	0	0	0
M5	Sarcoid	0	4	±	0	0	0	±	0
M6	Sarcoid	0	±	0	4	0	0	0	0
M7	Sarcoid	0	0	0	0	0	0	0	0
M8	Hemochromatosis	4	4	0	±	0	0	0	0
M9	Actinomycosis (involving liver)	4	4	4	0	0	0	4	0
M10	Ulcerative colitis	0	4	0	16	0	0	0	±
M11	Ulcerative colitis	0	±	0	0	0	0	0	0

* See Table 2 for key to abbreviations.

biliary cirrhosis (HB'1) and of 14 sera from seven cases of obstructive chronic biliary cirrhosis. Positive reactions to liver were encountered in two of the seven obstructive cases and to kidney in four of the seven cases. However, these were all of low titer compared with the consistently high titer reactions found in nine consecutive serum specimens collected over a period of six months from the one patient with primary nonobstructive chronic biliary cirrhosis. In biliary cirrhosis liver and kidney appear to be the screening antigens of choice.

Collagen Diseases.—The results of autoantibody complement-fixation tests with liver and kidney antigens on sera from patients suffering from various collagen diseases have been summarized in Table 1. Tables 6 and 7 present the titers with all eight human antigens for individual patients in each diagnostic category. Table 6 summarizes the limited data on acute rheumatic fever, rheumatoid arthritis, acute glomerulonephritis, and nephrotic nephritis (Type 2 nephritis) in adults, whereas Table 7 summarizes the more extensive data for cases of disseminated lupus erythematosus. It is apparent that low-titer reactions were occasionally encountered in the rheumatic diseases and nephritis, while in disseminated lupus erythematosus the reactions were positive with an impressively high frequency (liver 75%, kidney 83%) and in some cases at rather high titers.

Acute Rheumatic Fever: Only five cases of acute rheumatic fever have thus far been studied, but it is of interest that one of these, at least, has given positive reactions with liver antigen alone at titers of 16 and 4 in two successive serum specimens drawn six weeks apart.

Rheumatoid Arthritis: Only six cases of rheumatoid arthritis have been studied, and none have yet given positive reactions with liver antigen. However, three (50%) have reacted at low titers with kidney—two of these with muscle, two with thyroid, and one with spleen and heart in addition to kidney, muscle, and thyroid. Two specimens of serum in each of two positive cases gave rather similar reactions, although a third specimen in Case RA1, collected three months after the second specimen, was no longer positive.

Acute Glomerulonephritis: Two of the five cases thus far studied have reacted positively with liver and kidney antigens. In both these cases at least one serum specimen has shown CF activity with kidney, muscle, spleen, heart, thyroid, and adrenal antigens.

Nephrotic Nephritis: Only three cases have been examined. One has shown low-titer activity to liver antigen only and a second, with muscle, heart, thyroid, and adrenal but not with liver or kidney.

Disseminated Lupus Erythematosus: The results of the study with eight human antigens of 27 serum specimens from 12 patients with this disease are presented in Table 7. Although it is evident that muscle, spleen, and adrenal, and to a smaller extent heart and thyroid, antigens might be equally useful in detecting the AICF reagent in

TABLE 10.—*Summary of Reactivity of Exceptionally High-Titer Sera to Antigens Prepared from Human Adult, Human Embryo, and Rat Tissues* *

Case †	Diagnosis	Human Adult Tissue Antigens								Human Tissue Antigens	Embryo Antigens	Rat Tissue Antigens			
		L	K	M	S	H	P	T	A	EL	EB	RL	RK	RS	RH
MG1	Macroglobulinemia	16,000	2,000	128	8	16	16	64	512	1,000	128	8,000	4,000	16,000	16,000
		16,000	4,000	32	8	8	8	64	512	1,000	1,000	1,000	4,000	4,000	16,000
		8,000	2,000	2,000	8	8	16	256	1,000	1,000	256	2,000	512	4,000	16,000
		32,000	4,000	8,000	1,000	2,000	16	4,000	4,000	4,000	1,000	4,000	16,000	16,000	8,000
		32,000	8,000	8,000	8	8	8	4,000	2,000	16,000	256	32,000	2,000	4,000	32,000
MG2	Macroglobulinemia	512	1,000	2,000	64	1,000	0	128	256			2,000	512	1,000	
		1,000	1,000	1,000	0	0	0	±	±	0	0	0	0	±	256
HL1	Lupoid hepatitis	2,000	4,000	2,000	64	256	8	4,000	4,000	1,000	0	2,000	4,000	4,000	512
		>2,000	>2,000	256	32	16	±	1,000	512	2,000	0	1,000	128	128	1,000
		512	2,000	512	±	8	0	1,000	512	4,000	0	1,000	2,000	2,000	1,000
		1,000	2,000	1,000	0	1,000	0	512	4,000	2,000	0	1,000	4,000	64	1,000
		512	2,000	512	0	4	0	2,000	512	4,000	0	4,000	512	512	1,000
HL2	Lupoid hepatitis	64	16	8	32	8	0	8	16	4	32	8	16	64	16
HL3	Lupoid hepatitis	32	8	8	0	16	0	16	8						
HB1	Chronic nonobstructive biliary cirrhosis	64	128	64	0	128	0	128	128						
		1,000	4,000	16	8	128	0	32	256	2,000	16	2,000	4,000	4,000	2,000
		1,000	4,000	±	±	64	0	128	512	2,000	8	4,000	512	2,000	2,000
		2,000	4,000	8	16	128	0	64	512	2,000	8	2,000	2,000	4,000	2,000
		512	1,000	32	4	32	16	64	256	2,000	16	1,000	128	2,000	>2,000
		2,000	2,000	32	0	64	0	32	1,000	2,000	8	2,000	2,000	2,000	2,000
		1,000	4,000	128	8	256	8	128	512	2,000	8	2,000	4,000	2,000	2,000
		2,000	2,000	512	8	128	0	2,000	2,000			2,000		4,000	2,000
		4,000	4,000	64	4	128	0	1,000	256	2,000	32	1,000	2,000	4,000	2,000
L1	Disseminated lupus erythematosus	128	128	128	128	128	0	256	128	128	256	256	256	512	128
		256	256	128	128	128	0	256	64	256	256	>128	>128	>128	64
L2	Disseminated lupus erythematosus	32	64	8	8	0	0	16	16	64	32	4	8	64	0
		4	128	8	4	0	0	4	4	128	128	0	0	8	0
L10	Disseminated lupus erythematosus	±	4	0	256	0	0	0	4						
		±	4	±	256	±	0	0	4	0	0	0	0	32	0

* In addition to the abbreviations defined in the footnote to Table 2, the following abbreviations are used in this Table: EL, human embryo liver; EB, human embryo brain; RL, rat liver; RK, rat kidney; RS, rat spleen; RH, rat heart.

† Serial serum specimens are arranged chronologically. Dates of serum collection are listed in Tables 3, 5, 7, and 8.

disseminated lupus erythematosus, liver and kidney appear to be an adequate choice for serological screening. Sera from one patient, however, (L10) reacted much more strongly (titer of 256) with spleen antigen than with kidney and did not give a positive reaction with liver. Thus, it is not always sufficient to rule out the presence of high-titer human-tissue AICF reagents by using liver and kidney antigens alone.

Paraproteinemias.—Under the heading paraproteinemias, suggested in 1940, by Apitz,[4] are grouped the cases of myeloma and macroglobulinemia. They are both characterized by the presence in the serum of an abnormal globulin.

In Table 8 results of the study of sera from four cases of myeloma and five cases of macroglobulinemia with eight human antigens are presented.

Myeloma: Only one of the four patients with myeloma reacted with liver or kidney antigen, and this patient showed low titers of 4 with both antigens, as well as with muscle, heart, thyroid, and adrenal.

Macroglobulinemia: Two of the five cases of macroglobulinemia had high serum titers to liver antigen and to kidney antigen in serial serum specimens, one additional case gave a low-titer reaction of 8 with kidney antigen alone in one of the serum specimens, while the remaining two cases were negative with both antigens.

Other Diseases Giving Positive AICF Reactions.—Table 9 summarizes results with the group of sera from diverse diseases, not belonging specifically to the hepatic diseases, the collagen diseases, or the paraproteinemias, which have given positive reactions. These include primary syphilis, infectious mononucleosis, sarcoid, hemochromatosis, a case of actinomycosis of the liver, and ulcerative colitis. For none of these diseases have a sufficient number of cases been studied to warrant computation of the incidence of positive reactions. All positive reactions were of low titer to liver or kidney antigens or, as in the case of sera from infectious mononucleosis, hemochromatosis, and actinomycosis, to both. The sera from primary syphilis, infectious mononucleosis, and actinomycosis reacted similarly with muscle antigen. The case of primary syphilis had a strongly positive Wassermann reaction. A case of tertiary syphilis with tabes dorsalis with only ± Wassermann serology gave negative AICF reactions. Sera from one of two cases of ulcerative colitis and one of the three cases of sarcoid reacted with kidney antigen only.

High-Titer Sera.—Sera giving high-titer AICF reactions are of particular interest in that they provide the most favorable material for the study of differing organ specificities and for the characterization of the active serum reagent, or reagents, and of the tissue antigens with which they react. Certain high-titer sera have been intensively studied, and the results of these investigations will be reported in the third paper of this series.[6] In Table 1 the reactions of representative high-titer sera from nine patients with human adult and embryo antigens and with antigens from rat tissues are presented.

Cold agglutinin, heterophil antibody, Wassermann, Kahn, and Weil Felix (Proteus OX 19 and OX K) reactions and specific agglutination reactions to Salmonella typhi H and O antigens and Salmonella paratyphi A and B antigens, and to Brucella abortus were carried out on a number of these highly positive sera. Heterophil antibody titers on all these sera were within the normal range (i. e., below 64). One serum (HL1) with high-titer to human antigens showed a weak cold agglutination reaction, undiluted only. In no other instance were any of these reactions positive. Thus, this autoimmune CF reaction is not regularly associated with any of the above reactions, nor was it possible to associate it with positive cephalin flocculation tests.

Blood from two of the patients (HL1 and MG1) giving extraordinarily high titers was subjected to extensive hematological examination by R. J. Simmons, of the Commonwealth Serum Laboratories, Melbourne,

Australia. The red blood cells were grouped and typed as to ABO, MN, and Rh systems, and the sera were tested for agglutination or hemolytic activity against a battery of 10 different specimens of normal human erythrocytes as well as the patient's own cells. The specimens chosen represented all blood groups and MN genes, as well as Rh-positive and Rh-negative types. In no instances was there any activity. Direct Coombs tests for incomplete antibody performed on washed and unwashed red blood cells of the patient were negative.

In general, successive serial serum specimens from a given patient have yielded approximately the same pattern of reactions with the different organ antigens. However, there have been consistent differences in the spectrum of reactivity with different organs from case to case. Thus, sera from case HL1 of lupoid hepatitis have reacted consistently and repeatedly somewhat more strongly with kidney antigen than with liver antigen, while sera from a patient suffering from macroglobulinemia (MG1) have given the reverse pattern of organ preferential reactions, reacting more strongly with liver than with kidney antigens. Two serum specimens from patient L10, with disseminated lupus erythematosus, have reacted strongly with spleen antigens and only weakly, if at all, with other antigens.

Different batches of liver, kidney, thyroid, and adrenal antigens prepared from tissues obtained from different patients have yielded much the same reactions, but muscle, spleen, and heart antigens have been more variable in their behavior and their antigenic components have been less stable on storage. Results with pancreas are uninterpretable because of enzymatic action of the antigen on the CF reaction system, and therefore pancreatic antigen is no longer used.

These high-titer sera reacted at lower titers with antigens prepared from human lung, gastric wall, prostate, skin, peritoneum, pulmonary artery, and aorta than with human liver, kidney, muscle, thyroid, and adrenal antigens. However, certain interesting specificities appeared, as, for example, rather high-titer reactions of all sera from the macroglobulinemic patient, MG1, to lung and stomach antigens, while all serum specimens from a case of lupoid hepatitis, HL1, gave no reactions with these two antigens. Further investigations using these and other human antigens will be reported in a subsequent communication.[6]

Antigens prepared from normal human embryo tissue of an embryo of three months of gestation were used to titrate these strongly reacting human sera. Embryo liver, kidney, heart, brain, and skin muscle all contained active antigen. Antigen from embryo liver, kidney, and heart reacted with sera from patients MG1 and HL1 to give serum titers of 1000 or higher.

These high-titer human sera cross reacted with rat organ antigens at titers comparable to those with the human organ antigens. However, the pattern of preferential reactivity with different rat organs was often different from that observed for the human antigens. Thus, the sera generally titrated higher with rat spleen and heart antigens than with the corresponding human organ antigens. Furthermore, the titers with these organs from the rat were generally as high as with rat liver or kidney antigens. It is significant to note that the serum from the disseminated lupus erythematosus patient, L10, which reacted at high titer with human spleen antigen only, likewise reacted strongly only with rat spleen antigen and not with the other rat organs.

Characterization of the AICF Serum Reagents.—Before all titrations sera diluted 1:4 (or 1:8 and 1:16 in the case of a few high-titer sera) were heated at 56 C for 30 minutes. In Table 11 data on the heat stability of the serum reagent in high-titer sera from two patients (MG1 and HL1) are presented. Heating for 30 minutes at 60 C lowered the "autoantibody" titer to liver antigen by a factor of 4 and to kidney antigen by a factor of 8. Such heating at 65 C destroyed all activity in the macroglobu-

TABLE 11.—*Heat Stability of the Serum Reagents or "Autoantibodies"*

Serum Temperature,* Degrees C	Serum Titer			
	Liver Antigen		Kidney Antigen	
	MG1	HL1	MG1	HL1
55	32,000	2,000	4,000	4,000
60	8,000	512	512	512
65	0	32	0	128
70	0	0	0	0

* Temperature to which patients' sera were heated for 30 min.

linemic patient (MG1), while the serum reagent from the case of lupoid hepatitis (HL1) still titered 32 and 128 with liver and kidney, respectively. Exposure of this latter serum to 70 C for 30 minutes destroyed all activity. Thus, the heat sensitivity of the serum reagent is similar to that of many classical complement-fixing antibodies in human serum.

Serum from the first case of lupoid hepatitis (HL1) was submitted to paper electrophoresis. The electrophoresis was performed simultaneously on three parallel strips, using 0.02 ml. of serum per strip. The center strip was left unstained, while the two outer strips were stained and the pattern of protein distribution on them used to locate the proper position for cutting the central strip into five segments, representing albumin, α_1-, α_2-, β-, and γ-globulin, respectively. The serum proteins were eluted from these cut segments as moving boundaries by the capillary passage of the solvent (0.05 M sodium barbital buffer, pH 8.6). The protein in each segment was collected in 1.0 ml. of eluate, thus yielding a concentration of each component of the serum proteins in the eluate of one-fiftieth that in the original serum specimen. These fractions were then titrated against the antigens, and all the activity was found to reside in the slow-moving γ-globulin fraction.

Gamma-globulin precipitated from a number of positive sera appeared to possess all the activity of the sera. In one macroglobulinemic serum (MG1) the activity was demonstrated to reside exclusively in the eluate of the macroglobulin and γ-globulin on paper electrophoresis and not in similar eluates of other serum protein components. Furthermore, macroglobulin, three times reprecipitated by water dilution to low ionic strength, was found to contain all the activity of this extraordinarily high-titer serum. On the other hand, purified γ-globulin, from pooled normal human plasma, at concentrations exceeding the total γ-globulin concentration of most of our positive sera, did not react with any of the antigens.

Antigen Characterization.—All antigens have been titrated with use of high-titer postive human sera (i. e., MG1, MG2, HL1, HB'1, L1), as discussed in the section on materials and methods. It soon became evident that antigen titer depended on the serum chosen for titration because of different patterns of organ specificity from patient to patient. Furthermore, block titrations of antigen with potent antisera revealed distinct prozones in the region of low antigen and high serum concentration.

Liver and kidney antigen stored for two months at −70 C retained their activity, and once thawed they remained active with little loss of titer in 24 hours storage at icebox temperatures (4 C). At −20 C these antigens could be stored for one month with no appreciable loss of activity. Repeated freezing and thawing five times in rapid succession, with use of a −70 C bath (solid carbon dioxide [dry ice] and alcohol [ethanol]) for freezing and a water bath at 20 C for thawing, failed to lower the liver or kidney antigen titers significantly. Kidney antigen titer began to fall off after heating at 55 C for 30 minutes and disappeared almost completely on heating to 60 C for 30 minutes; however, at temperatures above 60 C the precipitation of denatured protein in the antigen made reading of the CF reactions difficult. Liver antigen was somewhat more heat labile, its activity with certain sera dropping significantly on exposure to 50 C for 30 minutes. Excessive precipitation of denatured protein, which interfered with the reading of the CF reactions, already appeared at 45 C.

Skeletal muscle, cardiac muscle, and spleen antigens were less stabile than kidney or liver on storage at −70 C or −20 C and within a few weeks often lost much of their activity. Adrenal and thyroid, on the other hand, were almost as stabile as liver and kidney antigens on such storage, and thus results with these four antigens were less variable than with the three less stabile homogenates. Enzymatic activity of pancreatic extract was so great that further study of its antigenic properties was not attempted.

Liver, kidney, and muscle antigens were submitted to two cycles of centrifugation at 35,000 g for 45 minutes, with resuspension of the first sediments in isotonic saline to the original volume. The sediments from the second spinning were found to contain virtually all of the antigenic activity of the homogenates, whereas the twice-spun supernatants contained very little activity and the washings, none. Similarly, the antigens in rat liver and kidney homogenates which reacted with the positive human sera were found to be sedimentable. These washed high-speed sediments contained essentially the mitochondrial and microsomal components of the cells contaminated with nuclear deoxyribosenucleoprotein threads, which are insoluble in isotonic saline.

Of particular interest were the high-titer sera from Patient HL1, with lupoid hepatitis, which all contained antibody in comparable titer against both the sedimented and washed particles of human-liver antigen and the unsedimented soluble components as well. The strong reactions of these sera with the twice-spun high-speed supernatants of liver antigen which failed to react at all with the other high-titer sera appear to be rather conclusive evidence for marked fundamental differences in the AICF antigens and their mutually dependent "autoantibodies" from person to person. The further characterization of the antigen components and of the complementary serum reagents is presented in the third paper of this series.[6]

Comment

It is generally accepted that species-homologous tissue components are not antigenic. Nevertheless, autoimmune mechanisms of pathogenesis have long been suspected in a number of pathological conditions, especially in those referred to as the "collagen diseases," which include rheumatic fever, rheumatoid arthritis, dermatomyositis, scleroderma, acute glomerulonephritis, periarteritis nodosa, cutaneous lupus erythematosus, and disseminated lupus erythematosus. Similar autoimmune mechanisms have been suggested for certain types of thrombocytopenic purpura, hemolytic anemia, acquired agranulocytosis, leukopenia or pancytopenia, encephalomyelitis, multiple sclerosis, amyotrophic lateral sclerosis, pemphigus, exfoliative dermatitis, Hashimoto's disease (lymphadenoid goiter), sympathetic ophthalmia, ulcerative colitis, and for certain forms of chronic hepatitis, including the so-called "lupoid hepatitis" recently described by Mackay, Taft, and Cowling.[5]

A vast literature has appeared on the experimental induction of immunological damage simulating the lesions of acute glomerulonephritis [7-20]; periarteritis nodosa [21]; rheumatic fever and rheumatoid arthritis [22-29]; acute disseminated encephalomyelitis, multiple sclerosis, and "diffuse sclerosis" [30-50]; disseminated lupus erythematosus,[24] and thyroiditis,[51,52] but the actual demonstrations of such immune mechanisms in cases of these diseases have been few and inconclusive. Only in the hematological diseases, such as the acquired hemolytic anemias, leukopenias, pancytopenias, and certain thrombocytopenias, have true circulating autoantibodies been demonstrated which are apparently of etiological significance.[53-57] A number of investigators have previously noted the presence of circulating antibody-like reagents reacting with antigens prepared from human tissues in some of the diseases of this group. A great variety of techniques for the identification of such "autoantibodies" have been used, but

no consistent methodology has evolved for the study of these autoantibody-like reagents occurring spontaneously in human sera. The numerous reports of such reactions, largely in European literature, have been reviewed recently by Schmidt [58,59] and Vorlaender.[60] Thus, tissue autoantibodies have been reported in rheumatic fever by Brockmann et al.,[61] Cavelti,[62] and Vorlaender,[63] in glomerulonephritis by Schwentker and Comploier,[64] Lange et al.,[65] Pfeiffer and Bruch,[66,67] in disseminated lupus erythematosus by Vorlaender,[68] and in liver disease by Eaton et al.,[69] Bjørneboe et al.,[70] Scheiffarth and Berg,[71] Scheiffarth,[72] and Vorlaender.[68]

Roemer et al.[73] have reported such complement-fixing antibodies in patients with multiple sclerosis, with use of an antigen prepared by alcohol extraction of cerebellar tissue of a patient who died of multiple sclerosis. In some patients suffering from Hashimoto's disease, Roitt et al.[74] have demonstrated a precipitin reaction between the patient's serum and purified thyroglobulin.

Woods and McPherson [75,76] demonstrated antibodies to normal uveal pigment in sera of patients suffering from sympathetic ophthalmia, and tissue sensitization to this pigment was demonstrated by positive skin reactions to pigment injected intradermally.

In pulmonary tuberculosis, Lehmann-Facius and Loeschcke,[77] as early as 1926, reported a specific precipitin reaction between patients' serum and saline extracts of organs, including the lung, and Fischer,[78] demonstrated a CF reaction and Aschkenasy,[79] a flocculation reaction, with use as antigen of an alcoholic extract of normal lung in which acetone-soluble lipids were the active antigens. Hippius [80] has more recently found such CF in tuberculosis, with use of human lung tissue extract as antigen.

Thomas et al.[81] noted in 1943 that patients convalescent from primary atypical pneumonia often develop in their serum the capacity to fix complement with a number of different antigens prepared from a variety of organs obtained from a number of unrelated species. They found that the antigens were easily sedimentable and that the serum reagents were inactivated by heating at 65 C for 30 minutes, two properties which they suggested utilizing to "circumvent the practical difficulties imposed by this property of certain convalescent sera" upon interpretation of the results of CF tests on acute convalescent serum pairs in virus disease diagnosis.

The positive findings with CF reported in this paper could be interpreted on classical lines as evidence for the production of antibodies against certain components of human tissue. Presumably such components might become antigenic either because of some modification of chemical pattern by viral, enzymatic, or toxic action or conceivably because components normally inaccessible to immunological processes have in some way been rendered accessible. The alternative interpretation is that we are dealing with an adventitious reactivity of serum globulins not arising as a specific response to tissue antigen.

It is not easy to decide between these two concepts on the available evidence. The serum reagent undoubtedly has many of the properties of typical antibody. It has been shown to be present in the globulin fraction of several positive sera. Its heat stability at 56 C, with increasing destruction above 60 C, is in line with the usual behavior of human complement-fixing antibody. So also is the presence of typical prozones in antigen titrations. On the other hand, the reproducible but at the present time inexplicable differences among sera in their reactivity with antigens from different tissues might seem to favor the view that the reactivity of the globulins in these cases is not immunological in origin. However, in experiments in this laboratory on rabbits, guinea pigs, and rats immunized by simultaneous intraperitoneal, intradermal, and subcutaneous injections of human liver antigen we subsequently found high-titer CF antibody responses to human kidney antigens of at least as high, and often of higher, titer than to the liver antigen which had been

injected. Also, rabbits and guinea pigs immunized with human liver or kidney suspensions developed CF antibodies to several other human tissue antigens (namely, muscle, adrenal, thyroid) and to rat liver, kidney, and spleen antigens as well. In some cases the titer to these other antigens, including those from rat tissues, exceeded that to the human immunizing antigen. Thus, the antigenic stimuli which presumably evoke the serum reagents we have detected are undoubtedly common to a wide range of mammalian tissue cells. Finally, the fact that the AICF reagents react with the human tissue antigens in a manner essentially similar to the reaction between such antigen and antibodies produced in rabbits, guinea pigs, or rats in response to injections with these antigens provides important confirmatory evidence that the reagents in our positive sera are true circulating antibodies.

Positive sera have generally been found in patients suffering from illnesses characterized by hyperglobulinemia. It has thus far been impossible to find significant correlation between positive reactions and any other factor in the serum of these patients than the alteration in the serum globulin patterns. In each disease wherein positive sera have been encountered, other sera not reacting in the AICF test have been found. This strongly suggests that the appearance of these reagents in the serum is not a matter of primary etiological significance but rather a secondary response to the pathological process. However, it is not possible to conclude from simple correlations of clinical findings with serum AICF titers whether or not "autoantibodies" are involved in the pathogenesis of these illnesses. Their absence in the serum does not vitiate the hypothesis of immunological "autoaggressive" pathogenesis. It is possible that other, nonhumoral, tissue antibodies, such as those responsible for homograft immunity,[82] which remain undetected by our technique are present and that such antibodies are of pathogenic significance. However, the presence of such autoimmune serum reagents provides strong evidence that disorders of immune mechanisms are associated with these nosological entities. The presence of these reactions almost exclusively in diseases wherein self-inflicted immunological damage has long been suspected to be of etiological significance justifies further study of such reactions in all clinical stages of illness in the collagen disease group and in other pathological entities suspected of being "autoaggressive" phenomena. Furthermore, we may speculate that, since any process leading to an abnormal destruction of cells might give rise to the conditions necessary for the evocation of such an autoimmune response, it might be reasonable to suspect these AICF reagents ("autoantibodies") to be found, at least in the low-titer range, at some time during the course of a wide variety of injuries which place upon the body the burden of eliminating destroyed tissue. Thus, the technique here described offers a means of searching for antibodies to human tissue components at various time intervals after such tissue insult as is encountered in burns, scalds, radiation injuries, frost injuries, trauma with fractures or crush injuries of soft tissues, myocardial infarction, and pulmonary embolism, and in cases of such long-standing tissue destruction as occurs in chronic necrotizing infections, such as actinomycosis, tuberculosis, lung abscess and osteomyelitis, toxic or viral hepatitis, and malignant neoplasms in which either somatic cells or the tumor cells themselves undergo extensive necrosis.

Summary

An autoimmune complement-fixation (AICF) reaction between human sera and human organ antigens used to detect complement-fixing reagents, presumably "autoantibodies," in the sera of patients suffering from a wide variety of acute and chronic diseases, is reported. Sera from 496 normal and diseased persons were studied. Antigens were prepared by centrifugal clarification of saline suspensions of normal

human liver, kidney, skeletal muscle, spleen, cardiac muscle, pancreas, thyroid, adrenal, prostate, lung, stomach, skin, peritoneum, aorta, and pulmonary artery, as well as from human embryo organs and pathological human tissues.

Of sera from 339 control subjects (187 normal persons and 152 patients suffering from miscellaneous diseases), 3% reacted with liver and 10%, with kidney antigens. Positive reactions to liver and kidney antigens were significantly more frequent than among these control subjects in sera from 157 patients whose illnesses could be divided into four nosological categories: hepatic diseases, collagen diseases, paraproteinemias, and a group of miscellaneous unrelated diseases giving positive reactions. These postive reactions were found to occur predominantly in association with diseases for which immune mechanisms of pathogenesis have long been suspected.

High-titer reactions, i. e., above 16, were encountered only in a few diseases, namely, acute viral hepatitis, lupoid hepatitis, primary nonobstructive biliary cirrhosis, disseminated lupus erythematosus, and macroglobulinemia.

Sera from certain patients revealed extraordinarily high-titer AICF reactions in the 1000 to 32,000 range. The magnitude of such titers was verified by starting titrations with quantitative 1:1000 dilutions of serum prepared by accurate volumetric technique. Such very high-titer sera occurred in two patients with macroglobulinemia, one case of primary nonobstructive biliary cirrhosis, and one case of lupoid hepatitis.

Sera from those diseases giving a high frequency of positive reactions with liver and kidney antigen were examined with eight human-organ antigens. Twenty to fifty per cent of patients with acute rheumatic fever, acute glomerulonephritis, or nephrotic nephritis showed positive reactions to kidney or liver or both, while seventy-five per cent of cases of disseminated lupus erythematosus reacted with liver and eighty-three per cent, with kidney. Serum from one of four multiple myeloma patients reacted with liver and kidney, while two of five (40%) patients with macroglobulinemia reacted with liver antigen and three of five (60%), with kidney. Diverse hepatic diseases all showed a high incidence of positive reactions. Thus, during the first two months of acute infectious hepatitis reactions to liver and kidney were frequent, and kidney reactions usually persisted into the third month. Over 75% of cases of chronic hepatitis and lupoid hepatitis reacted with both antigens, while only 63% of the cases of chronic nutritional hepatitis gave reactions with kidney and liver antigens.

Liver, kidney, thyroid, and adrenal antigens were more stable on storage and therefore gave more consistent reactions and usually higher serum titers than did antigens prepared from skeletal muscle, spleen, cardiac muscle, or pancreas. Results with pancreatic antigen were uninterpretable because of enzymatic activity. However, freshly prepared muscle antigen was particularly active with certain hepatitic sera, and other sera gave far higher titers with fresh spleen or muscle antigens than with either liver or kidney.

No strict organ specificity was encountered, nor were the AICF reactions species-specific, since high-titer positive sera reacted with rat-organ antigens at titers comparable to those obtained with human-organ antigens. The pattern of preferential organ reactivity differed markedly from patient to patient (e. g., serum from a patient with disseminated lupus erythematosus reacted strongly with spleen only and serum from a patient with lupoid hepatitis reacted more strongly with kidney than with liver antigen, while serum from a macroglobulinemic patient revealed the reverse pattern); however, all serum specimens from a given person showed, in general, the same spectrum of preferential reactivity.

Although it was not possible to prove that the AICF serum reagents are autoantibodies evoked by an antigen stimulus rather

than adventitiously reactive serum proteins, available evidence strongly suggests that they are classical antibodies. They have been associated with the γ-globulin fraction of high titer by sera by both precipitation and electrophoretic fractionation of serum proteins. Furthermore, they show prozones on antigen titration, their heat stability resembles that of many human complement-fixing antibodies, and they react very similarly to antibodies produced in rabbits, guinea pigs, and rats in response to injections with these antigens.

The antigenic components in the kidney, liver, and muscle extracts are almost completely sedimentable at 35,000 g for 45 minutes, with the notable exception of a soluble nonsedimentable antigen in human liver extract, which reacts with all serum specimens from one of the lupoid hepatitis cases to the same remarkably high-serum titers shown by these sera when titrated with crude liver antigen.

Macroglobulin reprecipitated three times by water dilution to low ionic strength from serum of one of the macroglobulinemic patients with high serum AICF titers contained all the activity of the serum, as did the fraction containing the slow-moving macroglobulin plus γ-globulin components on paper electrophoresis. Thus, it appears that the macroglobulin itself may be an immunologically active autoantibody in some patients.

The technique here described may be used as a tool in the investigation of the large group of diseases which have long been speculatively associated with immunological mechanisms of pathogenesis, as well as in the study of other types of tissue damage which may be expected to lead to autosensitization. When further clinical correlation has been made this test may offer diagnostic and prognostic guides to the clinician. Finally, the high-titer sera occasionally encountered in patients suffering from these "autoimmune" diseases may be used as analytic reagents for the characterization of tissue antigens involved in the reactions, or these sera may serve as starting materials for characterization of the active serum reagents, presumably "autoantibodies."

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An "Autoimmune" Reaction Against Human Tissue Antigens in Certain Acute and Chronic Diseases

II. Clinical Correlations

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The serum from a proportion of patients suffering from acute viral hepatitis, active chronic hepatitis, and other parenchymatous diseases of the liver, disseminated lupus erythematosus (D. L. E.) and other "collagen diseases"; macroglobulinemia and multiple myeloma, and certain other conditions has been shown by one of us [1,2] to contain antibody-like substances which fix complement in the presence of human tissue homogenates. This reaction has been designated autoimmune complement fixation (AICF). This serum activity could represent an adventitious chemical reaction not evoked by any antecedent antigenic stimuli, and it may be impossible in any given case to exclude this. Nevertheless, such an explanation has none of the heuristic value of the autoantibody hypothesis, and for the reasons given by one of us [2] we have adopted the view that these substances are autoantibodies which have developed as a result of the pathological antigenicity of some human tissue component.

For many years it has been commonly held that a number of disease conditions, including especially D. L. E., result from autoaggressive immunological action. The results of the AICF test in the diseases we have mentioned provide new evidence that some basic disturbance in immune mechanisms may be an essential feature in their pathogenesis. The present paper is concerned with the correlation between the results of the autoimmune complement-fixation tests and the clinical, biochemical, and histological findings in such conditions.

Material and Methods

Preparation of tissue antigens, details of the complement-fixation technique for the determination of AICF titers, and the results in some 500 individual sera tested against a variety of tissue antigens are described in the preceding paper.[2]

The present report is confined to the AICF test results with liver and kidney antigens in 50 selected patients, the majority of whom have been under regular observation in this unit for a considerable period of time. Clinical diagnosis had been confirmed by histological examination of biopsy specimens whenever possible. Details of laboratory procedures and biopsy techniques have been described in previous papers from this unit.[3-5] The L. E.-cell test * used was the Magath and Winkle modification [6] of the two-hour clot method. Autohemolysins were searched for by the method of Dacie and de Gruchy,[7] and autoagglutinins, by the method of Rosenthal et al.[8]; autoagglutinin titers were maximal at 4 C. The sensitized sheep-cell agglutination reaction of Rose et al.,[9] designated as Rose's test, was performed by the method of Alexander and de Forest [10]; titers of 1:64 or higher were considered significant.

The method of presentation which is followed has been to organize the data in a form which parallels that in which AICF reaction results were presented in the first eight Tables of the first paper of this series.[2] Thus the format, the sequential

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* The L. E.-cell test, the autohemolysin and autoagglutinin reactions, and Rose's test were performed in the Clinical Pathology Department of the Royal Melbourne Hospital, under the direction of Dr. D. C. Cowling.

TABLE 1.—*Complement-Fixation Reactions of Human Sera with Human Liver and Human Kidney Antigens*

Clinical Conditions	Subjects, No.	Titer 4->4, % Liver Antigen	Kidney Antigen
Normal	187	2	12
Miscellaneous diseases	152	3	7
Hepatic diseases			
Acute viral hepatitis	75	20	53
Chronic active hepatitis	9	78	100
Lupoid hepatitis	5	100	100
Chronic nutritional hepatitis	11	64	64
Chronic biliary cirrhosis	8	38	63
Collagen diseases			
Acute rheumatic fever	5	20	0
Rheumatoid arthritis	6	0	50
Acute glomerulonephritis	5	40	40
Nephrotic nephritis	3	33	0
Disseminated lupus erythematosus	12	75	83
Paraproteinemias			
Multiple myeloma	4	25	25
Macroglobulinemia	5	40	60

order of cases, and the code designations for individual cases used in that paper have been preserved, and each of the eight Tables of this paper corresponds to the correspondingly numbered Table in the former report.

Results

Table 1, abstracted from the first Table of the introductory paper of this series,[2] presents the number of cases studied and the percentage of positive reactions to human liver and kidney antigens in those diseases in which the incidence of autoantibodies exceeded significantly that found in the control subjects. Combining the results in 187 normal subjects and 152 subjects suffering with miscellaneous diseases in which the AICF reactions were no more often positive than in the control normal subjects, there were 339 control subjects, 3% of whom reacted with liver antigen and 10% of whom reacted with kidney antigen. As was discussed in the first paper, all of these reactions in the control subjects were of low titer (mostly 4 and 8), and only two exceeded titers of 16. These two "normal" sera reacted at titers of 32 with kidney antigen. The diseases wherein the incidence of positive reaction exceeded that in the control sera have been divided into four categories: hepatic diseases, collagen diseases, paraproteinemias, and a final group of 11 sera from diverse diseases which gave positive reactions.

Hepatic Disease.—This group has been subdivided into acute infectious hepatitis

TABLE 2.—*Acute Infectious Hepatitis (A.I.H.)*

Case Sex Age	Time Since Onset, Days	Titer with Human Antigens * L	K	Severity of Disease	Recovery	Remarks
HA1	8	±	±	++	Slow	Classical A.I.H.
M	34	0	4		complete	
25						
HA2	9	4	8	+	Prompt	Classical A.I.H.
F	17	4	4		complete	
32						
HA3	9	0	8	+	Prompt	Classical A.I.H.
M	29	0	0		complete	
30						
HA4	11	0	4	++	Slow	Diabetes mellitus; possibly chlorpromazine jaundice
F	17	0	0		complete	
65						
HA5	8-14	8	4	++	Slow	Classical A.I.H.
F	c.75	0	4		complete	
6						
HA6	8-14	0	16	+	Prompt	Classical A.I.H.
M	15-21	4	8		complete	
11						
HA7	8-14	0	8	+	Prompt	Classical A.I.H.
F	c.100	0	0		complete	
10						
HA8	8-14	±	4	++	Prompt	A.I.H.; associated cholelithiasis
F	c.56	0	±		complete	
27						
HA9	21	8	32	++	Prompt	Classical A.I.H.; hypergammaglobulinemia (1.7 gm. per 100 cc.)
M	30	0	±		complete	
20						
HA10	35	0	4	+	Protracted	Atypical A.I.H.
M	50	4	4			
18						
HA11	39	4	4	+++	Protracted	Classical A.I.H.
M	90	±	±		complete	
24						

* L indicates liver; K, kidney.

TABLE 3.—*Chronic Active Postviral Hepatitis* *

Case Sex Age	Date	Titer with Human Antigens L	Titer with Human Antigens K	Duration of Symptoms, Yr.	Chief Clinical Features	Severity of Disease	Progress	E.S.R. (Westergren) Mm./Hr.	L.E. Cell Test	γ-Globulin Level Gm./100 Ml.	Cephalin Flocculation	Cortisone Therapy	Significant Biopsy Findings
						Chronic Active Hepatitis							
HC1 M 18	10/9/56 12/11/56 1/9/57	8 0 8	8 16 ±	3	h+; s+++ thrombocytopenia, pupura, splenectomy on 12/10/56	+++	Static	82	— e,t,r	4.3	+++	Ineffective	Fibrosis ++, cellularity +
HC2 M 19	5/25/56 12/19/56	4 0	8 0	3	Asymptomatic, h++, s+++, n+	++	Static	101	— t	3.9	+++	Effective	Fibrosis ++, cellularity +
HC3 F 12	9/17/56	8	16	3	h++, s+, n+++	+++	Progressive; died 11/27/56	64	—		+++	Ineffective	Fibrosis +++, cellularity ++
HC4 F 48	9/12/56 1/3/57	4 ±	± 4	2	h+, s+, n+	++	Static relapsing	30	— r	2.3	++	Moderately effective	Fibrosis ++, cellularity ++
HC5 M 37	7/4/56 1/9/57	4 0	4 ±	6	h+, s+++, n+, thrombocytopenia	+	Improving	5	— e,t	1.5	+++	Effective	Fibrosis ++, cellularity +
HC6 M 32	9/13/56 12/17/56	4 0	4 0	6	h+, s++, n+	+	Static	9	— e,r	2.0	+++	Effective	Fibrosis +, cellularity +
HC7 F 23	10/19/56	±	4	10	Severe arthralgias, h++, n+	++	Static	7	—	1.4	—	Not given	Fibrosis +, cellularity ++
HC8 F 27	10/19/56 1/24/57	4 0	± 0	5½	h+, s++, n+, pancytopenia	++	Static	63	— e,t	1.6	++	Not given	Fibrosis ++, cellularity +
HC9 F 40	12/18/56	0	4	1	h+	++	Static					Not given	Fibrosis ++, cellularity ++
						Lupoid Hepatitis							
HL1 F 39	5/25/56 1/9/57	2,048 512	2,048 2,048	2	Severe arthralgias, h++, n++	+++	Static	88	++	3.0	+++	Effective	Fibrosis +, cellularity ++
HL2 F 66	8/–/56	64	16	4	h+++, s+, carcinoma stomach, diabetes mellitus, nephritis	++	Static; died 9/–/56	129	— (+ in 1955)	1.4	+++	Not given	Fibrosis +++, cellularity +
HL3 F 29	1/4/57	32	8	5	Facial rash, arthralgia, h++, n++, pancytopenia	+++	Deteriorating	99	— (+ in 1955)	2.7	+++	Not given	Fibrosis +++, cellularity ++, fat ++
HL4 F 20	7/6/56 10/9/56 12/12/56	4 ± 0	16 4 0	3	h+, n+	+++	Deteriorating; died 3/11/56	78	— (+ in 1955)	2.7	+++	Partially effective	Fibrosis +++, cellularity +
HL5 F 21	10/23/56 12/12/56	4 0	8 4	1½	Facial rash, h++, s+, n+	+++	Static	10	— (+ in 1955)	2.0	+++	Effective	Fibrosis +++, cellularity ++

* The following abbreviations are used in this Table and in Tables 4 and 5: h, hepatomegaly; s, splenomegaly; n, spider nevi; e, erythrophagocytosis; t, tart cells; r, rosettes.

TABLE 4.—*Chronic Nutritional Hepatitis* *

Case Sex Age	Date	Titer with Human Antigens L	Titer with Human Antigens K	Duration of Symptoms	Chief Clinical Features	Severity of Disease	Progress of Disease	E.S.R. (Westergren), Mm./Hr.	γ-Globulin, Gm./100 Ml.	Cephalin Flocculation	Significant Biopsy Findings
HN1 M 34	9/7/56	8	4	3 wk.	h++, n+	++	Static	46	1.1	+	Necrosis, fat, fibrosis +
HN2 F 40	9/17/56	16	0	4 wk.	h++, n++	+++	Improving	101		+++	
HN3 M 45	11/5/56 1/3/57	0 4	4 4	2 yr.	h++, n++, portocaval shunt 12/3/56	+++	Deteriorating	24	2.5	++	Fibrosis ++, cellularity +
HN4 M 46	12/14/56	4	4	10 yr.	h++	+	Improving		1.2	—	Fat
HN5 F 56	10/9/56	4	4	1 yr.	h++, chronic asthma	++	Chronic relapsing	107	1.9	+++	Necrosis, fibrosis +, fat
HN6 M 48	8/–/56	±	4	1 yr.	h++, glomerulonephritis	++	Improving	60	2.3	+++	Fat, cellularity +
HN7 M 53	11/2/55	±	0	2 yr.	h++	++	Static	18	1.4	++	
HN8 M 37	8/21/56 11/9/56	0 4	4 4	6 yr.	h++, s++, diabetes mellitus	++	Improving	33	1.2	+	Fat, cellularity +
HN9 M 53	11/16/56	±	0	6 yr.	h+++	++	Static	35	1.6	—	Fibrosis ++, fat ++, necrosis
HN10 F 41	11/12/56	4	4	4 wk.	h+, obesity	++	Improving	120	1.8	++	
HN11 M 40	2/27/56	±	0	1 yr.	h+	+	Improving				

* See Table 3 for key to abbreviations.

TABLE 5.—*Chronic Biliary Cirrhosis* *

Case Sex Age	Date	Titer with Human Antigens L	Titer with Human Antigens K	Duration of Symptoms	Chief Clinical Features	Severity of Disease	Progress of Disease	E.S.R. (Westergren), Mm./Hr.	γ-Globulin, Gm./100 Ml.	Cephalin Flocculation	Significant Biopsy Findings
					Primary						
HB1 F 39	8/8/56 9/21/56 1/4/57	1,000 2,000 4,000	4,000 2,000 4,000	36 wk.	Jaundice, pruritus, xanthomatosis, hepatomegaly	+++	Static	101	2.7	+++	Biliary cirrhosis, cellularity +++
					Obstructive						
HB'1 M 43	8/–/56 12/7/56	4 4	4 8	50 wk.	Stricture c.b.d., h++, s++, Coombs test positive	++	Improving	105	4.7	+++	Biliary cirrhosis, cellularity ++
HB2 M 71	11/26/56 12/3/56 12/17/56	0 ± 0	4 4 0	50 wk.	Cholelithiasis, h+, hepatic failure	+++	Recovered	133	2.0	+++	Diffuse hepatic necrosis
HB3 F 33	11/14/56 12/12/56	4 4	16 16	5 yr.	Stricture c.b.d., h+, impaired renal function	++	Static	53	1.4	—	Cholangitis, fibrosis +
HB4 F 41	11/12/56 12/8/56	0 0	4 0	2½ yr.	Stricture c.b.d., h+++	+	Static	71	0.9	+	
HB5 M 71	8/4/56	0	0	2 wk.	Cholelithiasis, h+, pernicious anemia	+	Recovered		0.8	—	
HB6 M 80	10/24/56	0	0	7 yr.	Carcinoma of pancreas, h+++	+++	Died		1.1	++	Biliary cirrhosis, fibrosis +++, cellularity +++
HB7 F 40	2/27/56	0	0	24 wk.	Cholelithiasis	+	Improving				

* c.b.d. indicates common bile duct. See Table 3 for key to other abbreviations.

Fig. 1.—Section of spleen, Case HC1—chronic active hepatitis and thrombocytopenic purpura. There is an exaggeration of the follicular pattern due to lymphocytic hyperplasia.

(Table 2), chronic active hepatitis including "lupoid hepatitis" (Table 3), nutritional hepatitis (Table 4), and biliary cirrhosis (Table 5).

Viral Hepatitis: Acute infectious (viral) hepatitis. The 11 selected cases of acute viral hepatitis illustrate the severity of the disease and recovery status of patients giving AICF responses during the acute and convalescent stages of the illness; slight to moderate activity characterized the icteric phase of the disease, and thereafter the reaction slowly became negative. Somewhat analogous complement-fixation and precipitation reactions have been previously reported with hepatitis sera and human liver antigen by Eaton et al.,[11] Olitzki and Bernkopf,[12] and Miles.[13]

Chronic active (postviral) hepatitis. Most sera from cases of chronic active hepatitis had a positive AICF reaction; in some cases having a low-titer reaction (HC2, HC4, HC5, HC6)† cortisone had been given for long periods and the disease was quiescent. Certain cases of chronic active hepatitis, termed "lupoid hepatitis," [5] showed L. E. cells. The clinical, biochemical, and histological features of these cases have been previously presented.[4,5] Significantly, they resembled D. L. E. in sex and age incidence and often exhibited other lupus-like lesions, such as arthralgia and a rash; arguments were presented [5] favoring an autoimmune pathogenesis rather than continuing viral action in this condition. In the other cases of chronic active hepatitis where no L. E. cells were found on repeated examination, rosettes, tart cells, and erythrophagocytosis were often detected; some of these patients also had possible autoimmune manifestations, such as arthralgia and cytopenias. The figures and data presented for the erythrocyte sedimentation rate (E. S. R.), L. E.-cell test, γ-globulin level, and cephalin flocculation test are representative of several analyses.

Case HC1.—An adolescent male with chronic active hepatitis had severe "hypersplenic" thrombocytopenia and purpura. The L. E.-cell test was negative,* but erythrophagocytosis, tart cells, and rosette formations were detected. Complement-fixing antibodies to liver, kidney, and muscle antigens were present in significant titer; it was reasoned

† Certain of the cases referred to and tabulated herein have been reported from this unit in other publications not directly concerned with the present study and are listed as follows: HC2, Case 6[14]; HC4, Case 2[14]; HC5, Case 1[14]; HC6, Case 4[14]; HL1, single case,[4] Case 7,[5] Case 5[14]; HL2, Case 4[5]; HL3, Case 3[5]; HL4, Case 1,[5] Case 3[14]; HL5, Case 2[5]; MG1, Case 3[15]; MG3, Case 2[15]; MG4, Case 1[15]; MG5, Case 4[15].

* A positive L. E.-cell test was obtained in October, 1957.

TABLE 6.—*Collagen Diseases* *

Case Sex Age	Date	Titer with Human Antigens L	K	Duration of Symptoms	Severity of Disease	Progress of Disease	E.S.R. (Westergren), Mm./Hr.	Remarks
					Acute Rheumatic Fever			
RF1 M 20	8/-/56 11/2/56	16 4	0 0	12 days	++	Recovered	62	Acute polyarthritis, A.S.T. 100
RF2 M 14	10/9/56 12/19/56	± 0	± 0	9 yr.	+++	Relapsing	64	Recurrent rheumatic carditis, A.S.T. 250
RF3 F 30	11/16/56	0	±	4 wk.	+	Recovered	45	Post tonsillitis; polyarthritis
RF4 M 35	1/16/57	0	0	2 wk.	+	Recovered	78	Migratory polyarthritis, A.S.T. 250
RF5 M 17	1/21/57	0	0	1 wk.	++	Recovered	90	Post tonsillitis; polyarthritis and cardiac bruits
					Rheumatoid Arthritis			
RA1 F 63	9/-/56 12/15/56	0 0	8 0	7 yr.	+	Mildly active	45	Generalized rheumatoid, diabetes mellitus, mild hypothyroidism
RA2 F 54	11/21/56	0	4	9 yr.	+++	Remission	10	Extensive rheumatoid, pulmonary tuberculosis
RA3 M 53	11/26/56	0	4	9 wk.	++	Active improving	40	Acute generalized rheumatoid; Rose test 256
RA4 M 38	8/-/56 11/16/56	0 0	0 0	12 yr.	++	Active	65	Relapsing generalized rheumatoid
RA5 F 65	11/28/56	0	0	20 yr.	+++	Active	13	Extensive rheumatoid, gastric ulcer
RA6 F 39	12/14/56	0	0	1½ yr.	+	Mildly active	18	Rheumatoid of hands and fingers
					Acute Glomerulonephritis			
NA1 M 43	12/4/56 1/9/57	4 ±	4 0	5 days	++	Slow recovery	95	Chronic asthma, classical acute nephritis
NA2 M 54	8/-/56 1/4/57	4 4	4 0	3 wk.	+++	Slow incomplete recovery	92	Alcoholism, acute nephritis following respiratory infection
NA3 M 41	12/12/56	0	0	12 wk.	+++	Death		Severe progressive acute nephritis
NA4 M 43	8/-/56	0	0	1 wk.	++	Recovered	26	Anuria following febrile illness—? sulphonamide sensitivity, A.S.T. 100
NA5 F 29	1/21/57	0	0	12 wk.	++	Static	131	Arthralgia, rash, edema, albuminuria—? disseminated lupus erythematosus
					Nephrotic Nephritis			
NN1 M 40	11/-/56	±	±	16 wk.	++	Static	102	Edema and heavy albuminuria, cortisone ineffective
NN2 M 55	8/30/56	0	0	2 yr.	++	Static	59	Cryptogenic nephrosis, cortisone therapy
NN3 M 45	8/-/56	4	0	5 yr.	+	Remission		Mild nephrotic syndrome, minimal tuberculosis

* L indicates liver; K, kidney: A.S.T., antistreptolysin titer.

that antiplatelet antibodies were present, but facilities did not exist to demonstrate them. Splenectomy was followed by an immediate rise in the platelet count. Microscopic examination of the spleen showed intense hyperplasia of the lymphoid follicles (Fig. 1).

Chronic Nutritional Hepatitis: The AICF reaction was less frequently positive in 11 cases of chronic nutritional hepatitis associated with alcoholism; these patients rarely had clinical findings suggestive of an autoimmune reaction. However, one female patient, HL3, with lupoid hepatitis was an alcoholic with severe liver disease; she had a facial rash, arthralgia, and a positive L. E.-cell test. The AICF reaction gave positive titers.

Chronic Biliary Cirrhosis: The AICF reaction gave negative results with liver antigen in five out of seven patients with obstructive biliary cirrhosis; one positive result may be correlated with the findings in this patient, HB1, of splenomegaly and a γ-globulin level of 4.7 gm. per 100 cc.

One patient, HB'1, a classical case of primary nonobstructive biliary cirrhosis, had highly elevated antibody titers. The obscure etiology of this condition renders this finding of considerable interest.

Collagen Diseases.—Data on the AICF reaction in rheumatoid arthritis, rheumatic fever, glomerulonephritis, and nephrotic nephritis are described in the preceding paper;[2] although certain sera in each of these groups gave positive reactions in low titer (Table 6), total cases are too few to attempt clinical correlations.

Disseminated Lupus Erythematosus: The D. L. E. group (Table 7) has been studied in the greatest detail from the clinical standpoint, since it is in this condition that antibody titers were most consistently elevated. Our 12 established cases illustrated well the protean manifestations of D. L. E.—arthritis, dermatitis, hemolytic anemia, purpura, pleurisy, nephritis, and endocarditis in various combinations. All but one had positive L. E.-cell tests on at least one occasion. Ten of these twelve cases of D. L. E. gave positive AICF reactions, but the height of antibody titers did not closely correlate with the acuteness of the disease, its clinical course, or the distribution of the lesions. Thus the highest titers, of 256, were obtained in a patient, L1, who was in remission with cortisone therapy except for persisting arthralgias; lower titers, of 4 and 8, were given by some of the more seriously ill patients—L5, L9, L10. Neither did tissue antigen specificity correlate with distribution of the lesions. Thus the serum of cases L1 and L8 reacted in high titer with kidney antigen, yet renal function tests were normal and renal biopsy showed no structural changes.

There was suggestive evidence (Cases L2, L6) that cortisone therapy suppressed the activity of the AICF test parallel with symptomatic improvement. Positive results were still obtainable, however, after protracted cortisone administration (Cases L1, L5), even when the L. E.-cell test had long since become negative. The two cases of

TABLE 7.—*Clinical Data on Cases of Disseminated Lupus Erythematosus* *

Case No.	Sex	Age	Total Duration of Symptoms (Jan. 1957)		History and Clinical Findings	Cortisone Therapy, Mg. (Duration)	Dates	L. E. Cell Test	Antigen Titers	
									Liver	Kidney
L1	F	42	6 yr.	H.:	Arthralgias & hemolytic anemia, 1951; splenectomy & cortisone induced remission, but arthralgias persist		Nov., 1951			
				C.F.:	Slight swelling & stiffness joints of fingers, wrists, ankles; uterine fibroids	50 (3 yr.)	Feb., 1954	+		
							Nov., 1955	†		
							Nov., 1956	†	128	128
							Dec., 1956		256	256
L2	F	56	1 yr.	H.:	20 yr. previously arthritis after tooth extraction; 1 yr. previously pain & stiffness hands & fingers, tiredness, purpuric rash, & weight loss	100 (from Nov. 8, 1956)	Nov. 1, 1956	+	32	64
				C.F.:	Cardiac bruits, palpable liver, purpura and ecchymoses, minimal joint changes fingers, B. P. 210/130; chest x-ray, pulmonary infiltrates; febrile course; casts, leukocytes in urine		Nov. 26, 1956	—	4	8
L3	F	26	32 wk.	H.:	Arthralgias, recurrent febrile pneumonias 6 mo.; severely ill 3 wk.; admitted in crisis	100 (from June, 1956)	June, 1956	++		
				C.F.:	Blotchy purpuric rash, face & arms; temperature 105 F; no other positive findings; incomplete remission with cortisone		Aug., 1956		8	8
							Sept., 1956		8	8
							Nov., 1956		8	16
							Feb., 1957	++	16	16
L4	F	25	3 yr.	H.:	Stiff painful joints 18 mo., hands, feet, knees; febrile episodes; facial rash	50 (from July, 1955)	July, 1955	+	4	8
				C.F.:	Lymph node enlargement, swollen interphalangeal joints, palpable spleen, cardiac bruit; arthralgias persist; otherwise in remission		Sept., 1956	++	4	16
							Nov., 1956	++		

L5 F 25	5 yr.	H.: C.F.:	Pains, stiffness in large joints, later in wrists & hands, & Raynaud's phenomenon, 5 yr.; recurrent pyrexia, pneumonia, & pleurisy, 3 yr. Enlargement of liver, spleen, lymph nodes; cardiac murmurs; scleroderma fingers, mouth; condition slowly progressive	100 (from Aug., 1954)	July, 1954 March, 1955 Oct., 1955 Sept., 1956 Oct., 1956 Dec., 1956	— + + —t,r —t,r	4 8 4	8 16 4
L6 F 10	16 wk.	H.: C.F.:	Swelling & stiffness right knee, 16 wk.; pain & stiffness ankles, elbows, wrists, 14 weeks; irritability, fever. Liver & spleen palpable, effusions knee joints, periarticular swellings ankles & left elbow; good remission with cortisone	100 (from Oct., 1956)	Oct. 16, 1956 Nov. 7, 1956 Nov. 20, 1956 Dec., 1956	++	8 4 ±	8 4 16
L7 F 41	21 yr.	H.: C.F.:	Facial rash & painful swollen wrists & elbows followed indolent finger infection, 1935; subsequently cardiac murmurs detected, purpura & bruising, pleuritic pains; Raynaud's phenomenon, arthralgias, & febrile episodes Facial telangiectasia, cardiac bruits, enlarged liver & lymph nodes, tendon nodules; splenectomy Feb., 1953; hyperplasia Malpighian bodies; disease now stable	100 (from March, 1954)	April, 1953 Jan., 1954 April, 1955 Sept., 1956 Dec., 1956 Jan., 1957	++ + + —r —	8 4 4	8 8 0
L8 F 22	8 wk.	H.:	3 mo. previously regarded as infectious hepatitis; recovered; 1 mo. later, multiple arthralgias, fever, erythematous facial rash, & pleuritic pains; dramatic response to cortisone with regression of all positive findings except periarticular swellings of fingers; remains in fair clinical remission	125 (from Aug., 1956)	Oct., 1956 Jan., 1957	++ ++	4 8	8 8
L9 F 34	7 yr.	H.: C.F.:	Raynauds phenomenon, 7 yr.; thereafter facial rash, cutaneous swellings, arthritis of elbow, lassitude, pneumonia; hypertension & albuminuria 5 yr.; recurring arthralgias, rashes, & edema legs, 2 yr. B.P. 210/140; liver, spleen, nodes not enlarged; gross albuminuria & poor renal function; death Sept., 1956	50 (from June, 1954)	June, 1954 Feb., 1955 April, 1956 Aug., 1956	++ — + +	8	8
L10 F 40	2½ yr.	H.: C.F.:	Thyrotoxicosis diagnosed 3 yr. previously; treated with thiouracil; bronchogram 2½ yr. previously followed by recurrent urticarial rash; 6 mo. previously, pyelitis, followed by lassitude & weakness; 1 wk., pleuritic pain, hemoptysis Palpable thyroid, cardiac bruit, splenomegaly, & erythematous lupus-like rash face & forearms	Not given	Dec., 1956	—r,e	±	4 (spleen 256)
L11 F 25	1½ yr.	H.: C.F.:	18 mo. red swollen fingers, facial rash, abdominal pain, arthralgias, & pneumonia Cardiac bruit, pleural effusion, effusions knees, femoral vein thrombosis, liver enlarged, spleen impalpable, heavy albuminuria; in remission at present	100 (from Nov., 1954)	Nov., 1954 Nov., 1956	++ —	0	0
L12 F 39	8 yr.	H.: C.F.:	7 yr. joint pains & fibrositis, lassitude Limitation movement hips, elbow, shoulders & spine; disease mild; in complete remission	75 (from Sept., 1955)	Aug., 1955 Dec., 1956	+ —	0	0

* e indicates erythrophagocytosis; t, tart cells; r, rosettes.

TABLE 7A.—*Clinical Data on Cases of Disseminated Lupus Erythematosus* *

Case Sex Age	γ-Globulin, Gm./100 Cc.	Cephalin Flocculation	Blood Urea, Mg./100 Cc.	Urea Clearance, % of Normal	Albuminuria	Histological Data	
						Liver Biopsy	Kidney Biopsy
L1 F 42	1.2 1.7 1.3 1.4	+++ +++ +++ +++	21		— —	Nov., 1951; slight fibrous tissue increase & collections of lymphocytes Oct., 1956; normal parenchyma, large lymphoid follicle (Fig. 2)	Feb., 1954; normal Oct., 1956; normal
L2 F 56		—	76		tr. +	Oct., 1956; normal	Oct., 1956; advanced glomerular lesions of D. L. E. with hyalinization; interstitial fibrosis
L3 F 26	1.9 1.8 2.2 2.1 1.9	+++ + ++ + +++	30	97	+ —		
L4 F 25	1.8 2.2	+++ +++	21	170	— —	Aug., 1955; normal	Aug., 1955; slight glomerular basement membrane thickening; no fibrosis or cellular infiltration; minimal lupus nephritis
L5 F 25	3.4 2.7 2.7 2.0	+++ +++ +++ +++	36 24	86 132	— —	July, 1954; architecture distorted by fibrosis; focal collections of lymphocytes and plasma cells—chronic hepatitis	July, 1954; normal Sept., 1955; basement membrane thickening & glomerular sclerosis; fibrosis & cellular infiltration
L6 F 10	2.9				—		
L7 F 41	1.1 1.4	—	15	62	— — tr.	Feb., 1953; normal Dec., 1956; fibrosis & lymphocytic aggregates in portal tracts	May, 1953; normal glomeruli; slight fibrosis & cellular accumulation in medulla May, 1955; hyaline thickenings in capsular & glomerular basement membranes; hyalinization, small vessels Sept., 1956; extensive fibrosis & cell infiltration; no progress of glomerular lesions
L8 F 22	1.6 1.3	— —	24	107	—	Oct., 1956; normal	Oct., 1956; normal
L9 F 34	0.7 0.6 1.1 1.0	— — — +++	78 60 137 369	27 20 22	+++ +++ +++	Sept., 1956; periportal fat, otherwise normal	June, 1954; considerable basement membrane thickening of glomeruli & hyalinization; interstitial fibrosis & large groups of lymphocytes Aug., 1955; severe diffuse glomerular changes Sept., 1956; gross interstitial fibrosis, tubular atrophy, & glomerular sclerosis
L10 F 40	2.7	+	34		—		
L11 F 25	1.4 1.1	+ —	93 46	85	++ +		March, 1955; swelling, increased cellularity, & hyalinization of glomerular tufts; patchy fibrosis & lymphocytic infiltration Oct., 1956; slight progression of lesion—hematoxylin bodies present
L12 F 39	1.1	—	30	112	—		

Case Sex Age	Laboratory Data							Rose Test Titer	E.S.R., Mm./Hr.	Total Serum Protein, Gm./100 Cc.	Albumin, Gm./100 Cc.	Globulin, Gm./100 Cc.
	Anemia	Neutropenia	Thrombo-cytopenia	Coombs Test	Hemaggluti-nins	Hemolysins	Wassermann Test					
L1 F 42	+	−	−	+	+	+	−		100	6.7	4.4	2.3
										6.2	3.6	2.6
				+				−	15	7.0	4.2	2.8
	−	−	−							6.0	3.3	2.7
									3			
L2 F 56	+	−	−	−					50	5.4	3.3	2.1
L3 F 26	−	−	+	+			−	−	99	7.2	3.2	4.0
									90	7.1	3.8	3.3
									51	6.7	3.8	2.9
									24	7.0	3.6	3.4
									29	6.6	3.0	3.6
L4 F 25	+	+	−	+	+	+	+	128	73	5.7	2.9	2.8
									56	6.4	3.3	3.1
L5 F 25	−	+		+	±	±	−	64	92	7.6	3.4	4.2
					+				75	7.9	3.5	4.4
				−	+		−	512	94	7.0	3.0	4.0
	−	−		−					82	6.2	3.8	2.4
									55			
L6 F 10	−	−	−	−			−		45	9.0	2.6	6.4
L7 F 41	+	+	+	+	+	−	−	−	70			
						+						
								−		6.9	4.0	2.9
	−	−	−						25	6.4	4.0	2.4
									60			
L8 F 22	−	−	−	−					35	6.4	3.7	2.7
				−					60	7.0	4.3	2.7
							−	−				
L9 F 34	−	+	−	−			−		52	3.7	2.0	1.7
				−	−	+	−	−	26	5.5	3.0	2.5
								−	37	6.1	3.4	2.7
	+	−	−						118	5.4	3.0	2.4
L10 F 40	−	+	−	−			−		110	7.1	2.1	5.0
L11 F 25	+	−	+	+	−	+	−		95	4.4	1.7	2.7
									49	6.3	3.4	2.9
								−				
L12 F 39	−	−	−	−	+	−	−	128	5	5.5	3.8	1.7

* tr indicates trace.

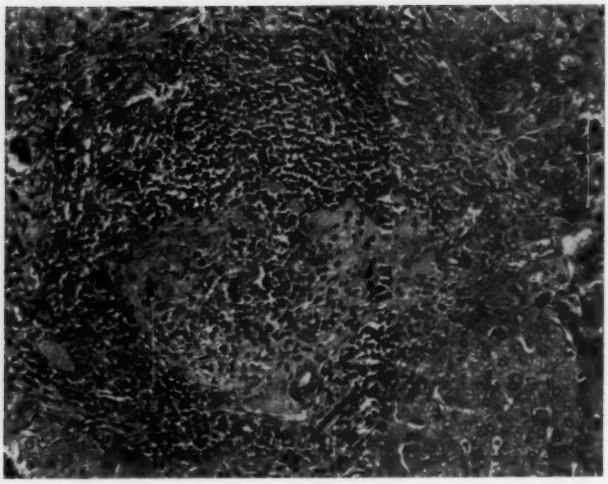

Fig. 2.—Section of material obtained by liver biopsy in Case L1—D. L. E. There is a large lymphocytic granuloma; the parenchymal cell pattern was normal.

D. L. E. giving negative tests (L11 and L12) were both in a cortisone-induced remission, although serial biopsies revealed progressive renal damage in one of them (L11).

No laboratory test (including the γ-globulin level) showed a close correlation with positive AICF titers, although there was usually some disturbance of such nonspecific indices as the E. S. R. and cephalin flocculation (C. F.) test. No false-positive syphilitic serology was obtained. A finding of possible significance in biopsy material from these cases was the frequent incidence of lymphoid collections, even when evidence of tissue damage was minimal. Thus Case L1, with consistently high AICF reactions, showed a large lymphoid granuloma in hepatic tissue obtained by needle biopsy,

TABLE 8.—*Paraproteinemia* *

Case Sex Age	Date	Titer with Human Antigens L	Titer with Human Antigens K	Duration of Symptoms	Clinical Features	E.S.R. (Westergren), Min./Hr.	Cephalin Flocculation	Mobility & Concentration of Abnormal Component, Gm./100 Cc.		Blood Findings and Bone Marrow
					Multiple Myeloma					
MY1 F 74	8/-/56	4	4	6 mo.	Lassitude, bone pains		+	γ	5.6	Severe anemia; B.M. plasma cells 39% †
MY2 M 53	10/-/56	0	0	6 yr.	Fractures, bone pains	120		γ	3.6	Unavailable
MY3 M 53	11/9/56	0	0	12 mo.	Fractured spine	100	—	γ	7.9	Mild anemia, leukopenia; B.M. plasma cells 50%
MY4 M 48	1/9/57	0	0	3 mo.	Bone pains, fractured sternum			Normoproteinemic		B.M. atypical plasma cells 95%
					Macroglobulinemia					
MG1 F 50	8/22/56 12/13/56	16,000 32,000	2,000 8,000	9 mo.	Lassitude, gross splenomegaly	92	+++	γ	2.2	Anemia, lymphocytosis; B.M. lymphocytes 56% ‡
MG2 F 76	10/-/56 1/8/57	512 1,000	1,000 1,000	18 mo.	Lethargy, ln+	56	++	γ	4.9	Anemia; B.M. lymphocytes 27%
MG3 M 55	12/12/56	0	8	5 yr.	Lethargy, bleeding, mental changes; h+, s++, ln+	103	—	β	4.8	Pancytopenia; B.M. lymphocytes 40%
MG4 F 56	11/-/56	0	0	5 yr.	Weakness, bleeding; h+, s+, ln++	73	—	γ2	5.8	Pancytopenia; B.M. lymphocytes 80%
MG5 M 50	11/-/56	±	0	15 mo.	Lethargy, bleeding; h+, s++, ln++			γ	5.0	Lymphatic leukemia; B.M. lymphocytes 80%

* L indicates liver; K, kidney; B.M., bone marrow; ln, lymph node.
† Autopsy MY1—diffuse plasmacytosis, lymph nodes and spleen.
‡ The histological diagnosis (lymph nodes, spleen) in Cases MG1, MG3, MG4, and MG5 was lymphosarcoma.

yet there was no evidence of diffuse parenchymal involvement (Fig. 2).

Paraproteinemias.—The group of "paraproteinemias" includes four patients with multiple myeloma and five patients with primary macroglobulinemia. Remarkably high titers were present in two of the patients with macroglobulinemia; it may be significant that both of these were females. Otherwise there was nothing in the clinical or laboratory data to distinguish these patients from two males and one female with macroglobulinemia who gave negative reactions; in particular, the serum globulin levels of the patients who reacted were of the same order as the ones who did not.

Other Diseases Giving Positive AICF Reactions.—Six patients with other diseases (Table 1, preceding paper [2]) gave positive AICF tests. We are unable to evaluate these reactions from the clinical standpoint until more patients with such conditions are available for study.

Comment

The results reported in this and the preceding paper [2] are sufficient to raise an almost endless series of questions, some of which could be subjects for immediate experimental study, others requiring extensive investigation at the clinical and laboratory level, and some concerned with processes of the internal coordination of bodily function which can be only tentatively formulated.

It has often been suggested that any process causing abnormal destruction of cells might under certain circumstances evoke an autoantibody response. On this hypothesis a wide variety of tissue injuries might be involved and an equally wide range of cell components develop pathological antigenicity. It is classical teaching that lipids released from cells damaged in the course of syphilis become autoantigens, evoking the production of the antilipid antibodies detected in the Wassermann and other syphilitic serological tests. Similar possibilities can be envisaged that cell proteins, nucleoproteins, polysaccharides, and particulate components, such as mitochondria and microsomes, may act as autoantigens if they are released by destructive processes from cell interiors into lymph or blood and so come into contact with cells of the reticuloendothelial system.

It is still a matter for speculation whether such cell components must be altered to become autoantigenic and if so what types of change are involved. Glynn and Holborow [16] have provided a suggestive demonstration in the conversion of tissue polysaccharide (chondroitinsulfuric acid) to autoantigen by union with a bacterial protein of Group B hemolytic streptococci. Gear [17] showed that whereas normal monkey liver was nonantigenic in the monkey, liver from a monkey at the height of infection with yellow fever contained antigens which on injection into monkeys produced antibody reacting with normal liver components. Numerous other experimental studies, particularly those of Cavelti and Rich and Gregory (cited by one of us [2]), have convincingly demonstrated autoantigenicity of homologous tissue components when either coupled with bacterial products or combined with adjuvants.

The self-marker hypothesis advanced by Burnet and Fenner [18] may be of value in this context. The physiological necessity that the body's own tissues should not provoke antibody production must be presumed to depend on the ability of reticuloendothelial macrophage cells to recognize autologous, or "self," material from "nonself." The ability for self-recognition may reside in specific marker components ("self-markers") in those expendable cells which are taken up by the macrophage system as the first stage of their disposal. This would hold typically for erythrocytes, lymphocytes, and granulocytes and probably for most other cells of mesenchymal origin. Where cell types are not normally taken up by the reticuloendothelial macrophage system, there is no physiological reason why they, or their components, should be

recognized as "self." Thus, either absence of self-markers on components from the interior of cells—or even from nuclear interiors—or their alteration, masking, or destruction by the action of enzymes, viral agents, or toxins might render such macromolecular components autoantigenic, and their contact with antibody producing cells of the reticuloendothelial system might cause autoantibody production.

The existence of such a mechanism would imply the possibility of disorders of its functioning being associated with or actually responsible for clinical disease. The production of antibody against the host's own tissue components, either in the form of circulating antibody or as cell-bound antibody of the type responsible for homograft immunity or tuberculin sensitivity, might be expected to produce symptomatic damage of various types. Pfeiffer [19] recalls that autoimmune mechanisms in disease were hinted at by Metchnikoff, rejected by Ehrlich, and reestablished by the demonstration of autoantibodies to erythrocytes in certain of the hemolytic anemias.

Of the large group of illnesses in which disturbances of immunological processes have been repeatedly observed, in the form of the production of abnormal antibodies or alterations of the reticuloendothelial system, disseminated lupus erythematosus may be selected as an outstanding example. D. L. E. classically runs a chronic fluctuating clinical course, with involvement of multiple organs and tissues in a progressive inflammatory process. Because of extensive involvement of the intercellular ground substance, or collagen, it has usually been classified with the "collagen diseases," a term which, as Klemperer [20] framed it, was purely descriptive, with no etiological implications. Rich and Gregory,[21] in attempting to simulate the lesions of rheumatic fever by experimental anaphylaxis in animals, produced lesions of the coronary arteries similar to those of rheumatic fever and D. L. E. The frequently false-positive syphilitic serology and positive Coombs test often found in D. L. E. gave further evidence of immunological disturbances. Vorlaender [22] had noted tissue autoantibodies in some cases of D. L. E. by the use of agglutination of tanned red cells treated with tissue antigens. This and the discovery of the L. E. cell by Hargraves [23] and the identification of the L. E. factor as a γ-globulin,[24] which suggested that the L. E. factor was an autoantibody to human leukocytes,[4] gave further support to the hypothesis that D. L. E. may have an autoimmune pathogenesis.

The presence of L. E. cells in certain other relapsing illnesses, such as rheumatoid arthritis [25] and the "lupoid" type of chronic active hepatitis,[4,5] seems evidence for a similar type of autoimmunological disturbance in these conditions also. Roitt et al.[26] have provided more direct evidence for such a pathogenesis by the demonstration of precipitin type autoantibodies in Hashimoto's thyroiditis.

We have found titers of complement-fixing antibodies to human tissue components more consistently elevated in D. L. E. than in other collagen diseases, for which data are, at present, rather limited. However, even in D. L. E. it has not been possible to correlate antibody titers with such clinical phases as the onset, acute relapses, distribution of the lesions, and spontaneous or cortisone-induced remissions. Moreover, titers bore no direct relationship to the L. E.-cell phenomenon, being often significantly high even when the L. E.-cell test had long since become negative. This would suggest that despite clinical evidence of remission some immunological disturbance was still operative. All diseases of the collagen-disease group, including rheumatic fever and rheumatoid arthritis, have long been considered as multisystem disease processes, and even chronic hepatitis is now being recognized as an illness which may involve the kidney and other organs as well as the liver.[5] Thus, if circulating antoantibodies are in fact themselves related in any primary or secondary way to such disease process, it should not be surprising

that we have found antibodies against antigens from a wide variety of organs in almost every case wherein any AICF activity was detected.

The fact that in two cases of macroglobulinemia the abnormal globulin was immunologically reactive but that it was not so in the other three opens up a wide field for experiment and speculation. Mackay et al.[15] suggested that the basic process in this condition is frequently a lymphosarcoma, with macroglobulin synthesis the result of an associated somatic mutation in the tumor-cell line. If this is so, the additional postulate must be added that the mutation may regularly or occasionally involve some essential phase of the mechanism of immune γ-globulin production. In view of this, cases of macroglobulinemia may be of great service in elucidating why autoimmune reactions are demonstrable in some persons but not in others with similar clinical conditions.

Some of the implications of the autoantibody hypothesis may be discussed in relation particularly to hepatic disease, which has been a major interest of this unit for many years. A moderate transient AICF activity often occurs in acute viral hepatitis and occasionally also in nutritional and obstructive hepatitis. Persistence of this activity may accompany transition of acute hepatitis to the chronic stage. We previously suggested[5] that certain cases of chronic active "lupoid" hepatitis may have an autoimmune pathogenesis; this concept was based on the resemblance, even to the presence of L. E. cells in the blood, between lupoid hepatitis and D. L. E., a condition in which autoimmune mechanisms have long been suspected. However, it now appears that lupoid hepatitis may represent an artificial division, since other "non-lupoid" cases have also had arthralgias, marked hypergammaglobulinemia, and probable autoimmune cytopenias, as well as rosettes, tart cells, and erythrophagocytosis in the L. E.-cell test. Moreover, AICF titers have been significantly elevated, not only in lupoid hepatitis but in cases of chronic active hepatitis as well.

If we accept Burnet and Fenner's[18] concepts of antibody production, the conditions under which autoantibody production would be expected to occur in diseases such as hepatitis are (1) modification of immunological pattern in cell components by viral, enzymatic or toxic damage; (2) interaction as a result of a disease process between specialized not normally expendable cells and the macrophage system; perhaps more specifically stated, between cell components lacking self-markers and the antibody-producing system.

In relation to infective hepatitis and its complications there are two main possibilities to be considered. The first is a modification of antigenic pattern by viral damage analogous to that demonstrated by Gear[17] in monkey yellow fever. The second is that at various stages of the infectious process and its sequelae damaged cells and/or their components may reach the antibody-producing system and in view of their absence of self-markers provoke autoantibody production. The closest experimental model is the autoimmune thyroid disease induced in rabbits by immunization with saline extracts of autologous thyroid tissue, described by Rose and Witebsky.[27]

Whatever the mechanism, the findings in the course of acute viral hepatitis suggest that the AICF reaction develops in approximately 50% of cases and that in the great majority who make a complete recovery the reaction disappears completely. Higher titers and persisting reactions are characteristic of cases of both chronic active and lupoid hepatitis. To date it has not been possible to follow the serological changes in individual cases over the whole period from the acute attack to the development of the chronic condition. Such a sequence of tests would obviously be of the greatest interest in interpreting the natural history of chronic hepatitis and in deciding why a minority

of the persons who suffer from acute hepatitis undergo transition to the chronic stage.

In considering such problems we believe that the following two points concerning immunity should be kept in mind.

1. If acute cell damage as in hepatitis provides opportunity for the development of autoantibodies, this process must be of extremely common occurrence and in the great majority of people of no harmful significance. If, as is reasonable to expect, it may also follow trauma or local infection, it becomes a physiological necessity that there is some mechanism by which autoimmune reactions are prevented from becoming self-perpetuating or productive of symptoms.

2. If damaged cells (e. g., of the liver in hepatitis) are taken up by the antibody-producing system, numerous possibilities exist in regard both to the specificity of the antibody produced and its qualitative type. In this study we have been concerned chiefly with a circulating antibody which fixes complement with a microparticulate fraction of liver and kidney cells. From the recent work of Billingham, Brent, and Medawar [28] it would seem most probable that nuclear components of the cells would provoke "homograft immunity" which is not associated with soluble antibody but with what can be called "cell-bound antibody." Such nonhumoral tissue immunity seems more likely, as has been noted above, to possess pathogenic potentialities than the circulating autoantibodies which we have demonstrated.

It may be that the real basis of the clinically evident and usually progressive character of diseases in the group of conditions that show autoimmune reactions is a failure of some protective and almost homeostatic mechanism which normally functions to prevent the ill effects of autoantibody production. In this way a self-perpetuating disease condition could be engendered. The fact that the lupus process not only may arise *de novo* but also may follow conditions such as hepatitis and malignancy (six such cases were reported by Lansbury [29]) is consistent with the autoimmune hypothesis.

Uncertainty exists as to how closely the normal tissue antigens employed in the present studies correspond to the autoantigens which must be presumed responsible for production of the antibodies with which we have been concerned. Even more important, there is as yet no direct evidence or even likelihood that this circulating antibody as such is responsible for the tissue damage which defines a patient as suffering from D. L. E. or lupoid hepatitis. Everything points to a more fundamental immunological anomaly as a background on which the various lupus manifestations—including, in addition to the present AICF reaction, positive L. E.-cell tests, Coombs reactions, and disseminated tissue damage—can develop more or less independently. It will be a task for the future to determine which cellular components are antigenic in any given case, what range of antibodies is present, and how damage to the tissues involved has been produced.

At the present time this attempt to be more precise about the immunological status of these conditions is purely speculative, but it does suggest fruitful lines of experimental approach, particularly in regard to D. L. E., where there are only slender clues to the mechanism by which the autoimmune state is initiated. We believe that autoantibody studies such as we have described will provide further insight into the nature of the lupus process, with the result that less meaningful concepts such as "collagen disease" will become obsolete. Moreover, the antibody studies described in these papers may prove to be a further valuable diagnostic aid in D. L. E. and related disorders.

Summary

A substance has been demonstrated in human serum which fixes complement in the presence of human tissue homogenates; this substance is presumed to be an autoantibody, and the reaction is designated autoimmune complement fixation (AICF).

The results of tests using liver and kidney antigen are presented for three categories of disease: hepatic diseases, collagen diseases, and paraproteinemia.

In acute viral hepatitis, low-titer positive reactions become negative coincident with recovery. Most sera from cases of chronic active hepatitis reacted in low titer; higher titers were obtained in chronic active hepatitis with L. E. cells—"lupoid hepatitis." Groups of patients with nutritional hepatitis and obstructive biliary cirrhosis showed a somewhat lower incidence of reactivity.

A high proportion of patients with disseminated lupus erythematosus had raised AICF titers, but this finding could not always be correlated with the clinical progress or the organs most severely involved, the L. E.-cell phenomenon, or other laboratory data.

Sera from two out of five patients with primary macroglobulinemia reacted in extremely high titer, but there were no corresponding clinical differences in these two patients.

We have postulated that this abnormal serum reactivity may characterize transition of acute hepatitis to the chronic stage and may also be related to the basic disease process in conditions such as D. L. E. The circulating autoantibody itself is not considered responsible for tissue damage or disease; we rather believe that the AICF phenomenon reflects a more fundamental immunological anomaly involving also the nonhumoral or "cell-bound" antibody system.

Sir Macfarlane Burnet helped to develop the immunological concepts expressed in this paper. Miss Lois Larkin gave valuable technical assistance, Dr. Leon Taft interpreted the histological material, and Miss Sara Weiden performed the biochemical investigations. Numerous physicians on the staff of the Royal Melbourne Hospital allowed us access to their patients.

The Walter and Eliza Hall Institute of Medical Research, c/o Royal Melbourne Hospital Post Office.

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Adenovirus Vaccine

Development, Field Evaluation, and Appraisal of the Need for Vaccination in Military and Civilian Populations

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Background

The acute respiratory illnesses caused by adenoviruses (RI-APC-ARD)[1-3] constitute a major problem in military medicine. These agents are the cause for the majority of cases of acute respiratory illness among new recruits in the Armed Forces.[4-8] By contrast, adenovirus-caused respiratory disease is relatively infrequent among the "seasoned" cadre, and this is true also of the adult civilian population.

The respiratory illnesses caused by adenoviruses fall into the general syndromes of febrile catarrh, undifferentiated acute respiratory disease (ARD), nonstreptococcal exudate pharyngitis, atypical pneumonia unassociated with the development of cold agglutinins, and pharyngoconjunctival fever.[9-11] More commonly, these illnesses are referred to as catarrhal fever, grippe, acute pharyngitis, "severe colds," or virus pneumonia. The adenoviruses have not been associated etiologically with the common cold.[9,12] At least 14 distinct immunological types of adenovirus have been recognized up to the present.[13] Only three of these, however, namely, Types 4, 7, and 3, have been associated with epidemic respiratory disease in the United States. In the Armed Forces, Type 4 is most important, Type 7 is less so, and Type 3 is the least frequent cause for respiratory illness.[7-9,14]

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Adenovirus Vaccine Development

The attack rates for hospital cases of adenovirus-caused respiratory disease in epidemics among new recruits in the winter may be as great as 20%,[4,5] resulting in great loss in military manpower, in serious disruption of the troop-training program, and in high costs for medical care and repeat training. This has made it imperative to develop an effective vaccine against the adenoviruses for use by the Armed Forces. Investigations to develop and evaluate such a vaccine have been in progress in our own and other laboratories, and highly significant advances have been made.

Two vaccines have been developed to date. The vaccine developed by Hilleman et al.[15-17] at the Walter Reed Army Institute of Research is a bivalent material containing Types 4 and 7 adenovirus, the predominent military strains. The virus for vaccine production is grown in tissue cultures of monkey kidney maintained in protein-free synthetic Mixture 199. The infectivity of the virus is destroyed by treating with 1:4000 formalin at 36 C. The sterility of the preparation is tested on a number of artificial media, and the safety is assayed in tissue cultures and in a variety of mammalian hosts, including monkeys. The potency is assayed by measurement of neutralizing antibody response after injection into guinea pigs and man. In man the vaccine induces a high level of neutralizing antibody against homologous Types 4 and 7 virus and against heterologous Type 3 virus as well, which is as great as that which follows the natural disease. The vaccine developed by Huebner et al. at the National

Institutes of Health[18] and prepared commercially for field trial[19] is a trivalent material containing Types 3, 4, and 7 adenovirus. The virus in the infected monkey-kidney suspension is inactivated by treating with 1:400 formalin, and the formaldehyde is partially neutralized by treatment with bisulfite. The assays for safety and potency are carried out in tissue cultures and experimental animals.

Vaccine Evaluation in Controlled Field Studies

The bivalent Types 4 and 7 vaccine developed by Hilleman et al.[15-17] has been evaluated in two controlled field studies in newly recruited soldiers. The trivalent Types 3, 4, and 7 vaccine of Huebner et al.[18] was tested in human volunteers who were challenged with live virus inoculated onto the conjunctiva and was later evaluated in a controlled study among naval recruits.[19] The present paper summarizes the results to date of the field evaluations in epidemic recruit disease.

Vaccine Trials (1956).—The first evaluation of the bivalent vaccine was carried out in newly recruited soldiers at Fort Dix, N. J., during the winter of 1956.[15,17] The vaccine employed was prepared in this laboratory. The plan of study was simple and was designed to provide answers to two basic questions: First, is the vaccine effective, and second, if so, to what extent? The study group was limited to a size small enough to insure close regulation and accurate observation but was much larger than required to give valid statistical results. In the study, 311 men chosen at random from six newly formed recruit companies were given two 1 ml. doses of vaccine intramuscularly, one week apart. At the same time, a second group of men, similarly chosen, was given formalin-saline placebo for control purpose. The evaluation of the vaccine effectiveness was made in terms of (*a*) a comparison of the hospital-case attack rates for respiratory illness, all causes, in the two groups and (*b*) the attack rates for adenovirus-caused respiratory illness, only, in the vaccinated and control persons based on the results of laboratory serodiagnostic tests. The period of the study was approximately three months. The vaccine effect became apparent seven days post vaccination, and all results were expressed in terms of the cases which occurred after this time. Only a few respiratory-disease cases occurred prior to the eighth day. During the time of the study, roughly 82% of the respiratory cases among nonvaccinated persons admitted to the hospital were caused by adenovirus. Types 4 and 7 adenovirus cases were present in the epidemic in approximately equal number.

Findings in the evaluation at Fort Dix are summarized in Figure 1. The attack rates for hospital cases of adenovirus-caused respiratory illness was 19.5% for the controls and only 0.3% for the vaccinated. The protective ratio resulting from vaccine use was 1:61, the vaccine causing a 98% reduction in the expected number of cases. The nonadvenovirus attack rates in the two groups were each 4.2%.

The over-all effectiveness of the adenovirus vaccine in reducing the incidence of total respiratory disease, all causes, is, of necessity, related to the proportion of cases in the epidemic caused by adenovirus. In this epidemic, as stated above, approximately 82% of the cases in nonvaccinated persons were caused by adenovirus. The attack

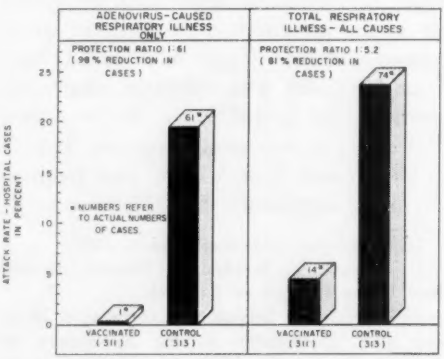

Fig. 1.—Summary of evaluation of bivalent Types 4 and 7 adenovirus vaccine, Fort Dix, N. J., 1956.[17]

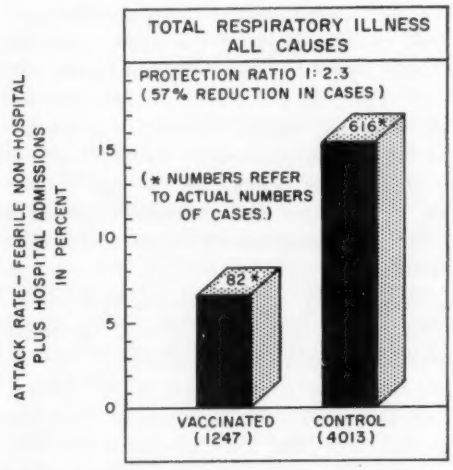

Fig. 2.—Summary of evaluation of trivalent Types 3, 4, and 7 adenovirus vaccine, Great Lakes, Ill., 1956.[19]

rates for total respiratory disease, all causes, in the vaccinated and control groups were 4.5% and 23.6%, respectively. The over-all protection ratio was 1:5.2, and the reduction in expected occurrence of respiratory disease, all causes, was 81%.

Evaluation of the trivalent Types 3, 4, and 7 adenovirus vaccine was carried out at Great Lakes Naval Training Center during the winter of 1956. A summary, compiled from a preliminary progress report by Bell et al.,[19] is given in Figure 2. This covers the findings during an approximate three-month period (Jan. 14-April 14) and represents only one-third of the total experience. The findings are given in terms of attack rates for febrile hospital plus nonhospital cases of respiratory illness, all causes, which occurred two weeks or longer post vaccination. In the epidemic, the attack rates for total respiratory disease were 6.6% for the vaccinated and 15.4% for the controls, giving a 57% reduction in expected incidence of total respiratory disease among vaccinated persons.

It is to be noted that the evaluation of bivalent vaccine at Fort Dix was carried out during a period of high incidence of adenovirus-caused respiratory disease, when the adenovirus-specific rate was 19.5%, and 82% of the respiratory disease cases among nonvaccinated persons were caused by adenovirus. In the study of trivalent vaccine at Great Lakes, the adenovirus-specific attack rates were not recorded, but it is seen that the total respiratory disease attack rate among nonvaccinated personnel on this post (15.4%) was less that that for adenovirus-specific disease only at Fort Dix (19.5%). In the absence of the adenovirus-specific rates for Great Lakes, no definitive comparison of the relative effectiveness of the bivalent vaccine with the trivalent vaccine can be made.

Vaccine Trial (1957).—The second field evaluation of the bivalent Types 4 and 7 adenovirus vaccine was carried out during the winter of 1957 by Hilleman et al.[20] in collaboration with the personnel of the U. S. Army Hospital at Fort Leonard Wood, Mo. The vaccine employed was prepared under commercial conditions by the Lederle Laboratories Division, American Cyanamid Company. The evaluation, incomplete at the time

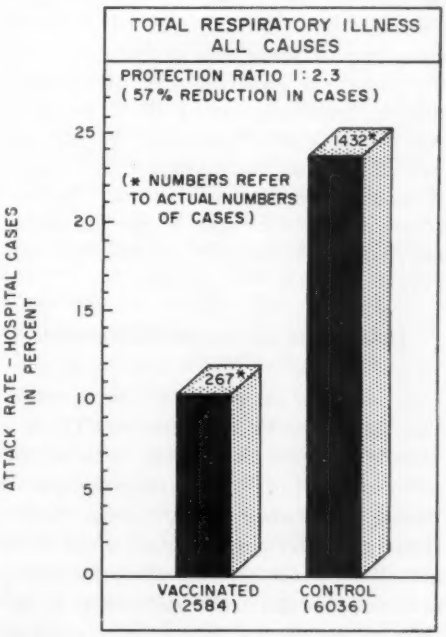

Fig. 3.—Preliminary evaluation of bivalent Types 4 and 7 adenovirus vaccine, Fort Leonard Wood, Mo., 1957.[20]

of this writing, is summarized in preliminary form in Figure 3. In the study, approximately 2584 new recruits were given a single 1.5 ml. dose of vaccine, and 6036 men received a like amount of formalin-saline placebo within 36 hours of arrival on post. Evaluation of the vaccine was expressed in terms of the reduction of hospital cases of respiratory disease, all causes, and in terms of an estimate of the reduction in adenovirus-caused cases, specifically, based on serodiagnostic tests of a sample of the cases. In the epidemic, roughly 70% of the respiratory illness among nonvaccinated persons was caused by adenovirus. Virus recoveries from patient's throat washings indicated that Type 4 adenovirus only was present in the epidemic.

The attack rates for respiratory illness, all causes, among the vaccinated and controls were about 10.3% and 23.7%, respectively, giving an approximate protection ratio of 1:2.3 and a reduction in expected incidence of respiratory illness, all causes, of approximately 57%. It was estimated, based on serodiagnostic tests of a sample consisting of about 800 of the cases, that the protection ratio for adenovirus-caused disease, specifically, was 1:10. Thus, there was about a 90% reduction in expected incidence of adenovirus-caused respiratory disease as a result of vaccination. The non-adenovirus attack rates in the vaccinated and control persons were roughly 9% and 7%, respectively.

Indications for Use of Adenovirus Vaccine

Definition of the Problem.—Acute respiratory illness caused by adenovirus Types 3, 4, and 7 appears, in general, to be of low prevalence and of sporadic occurrence in its "normal" existence in the human family. However, under circumstances which bring into close contact large numbers of susceptibles, this "normal" relationship may be upset and the disease may assume epidemic proportions. Any attempt to appraise the need for adenovirus vaccine in a particular population group must be viewed, therefore, from the standpoint of the attack rates, the chance that the particular vaccine used will be effective in preventing disease, and the socioeconomic implications of the occurrence of the disease. Unless the factors are kept in their proper perspective, the unwise decision to use vaccines in situations which offer little or no chance for significant benefit may result.

Military Use.—The importance of adenovirus-caused respiratory illness in newly recruited soldiers is exemplified by the results of a study [4] conducted by this laboratory at Fort Dix, N. J., during the one-year period from June, 1954, through May, 1955. During this time, roughly 60,000 recruits were given basic training on the post, and this represented the population at risk. Among the trainees roughly 6000 hospital cases of adenovirus-caused respiratory illness occurred, and the men were confined in the hospital for an average period of about eight days. Thus, roughly 1 of every 10 soldiers who trained at Fort Dix during the year was hospitalized for adenovirus-caused respiratory disease. The total cost for time loss and for hospital care only was estimated to be well in excess of $1,500,000 at this one Army post. The high attack rate at Fort Dix is generally consistent with the pattern of occurrence in other recruit training bases in the United States.[6-8,21] The great importance of the adenovirus vaccine to the military recruit group is without question, and the general use of the vaccine in such persons shows promise of eliminating adenovirus infection as an important medical and economic problem to the Armed Forces. The value of the vaccine, however, is even greater during periods of mobilization for war, when large numbers of men are being inducted into the Armed Forces and the impact of non-effectiveness from disease is most serious.

In striking contrast to that in recruits, the incidence of respiratory illness caused by adenoviruses in the cadre population, or "seasoned" military group, is very low. In

the study at Fort Dix cited above, the percentage of hospital cases of respiratory disease caused by adenovirus in such personnel was 15%, and the attack rate was very low, being about 1% for the year. In other camps, such as Fort Meade [4] and elsewhere,[21-23] which have no prominent recruit training activity, the occurrence of adenovirus disease is, indeed, infrequent. On the basis of this information, it is concluded that there is no real need for adenovirus vaccine among cadre personnel, and use of the vaccine in such populations would not be indicated.

Civilian Use.—While much definitive information is available relative to the incidence of adenovirus-caused respiratory illness in military groups, similar extensive data are not presently available for the civilian population. The evidence published to date consists of descriptions of a few sharply defined outbreaks which occurred principally in children,[11,24-28] several studies of selected patients with respiratory illness to determine the proportion of the cases caused by adenovirus,[22,23,29-34] and several longitudinal seroepidemiological investigations to determine the adenovirus infection rate over extended periods of time [31,34,35] in certain population groups. These investigations generally failed to define the population at risk and, thus, permitted only a rough guess of the importance of adenovirus in civilian respiratory illness.

In general, however, all evidence to date indicates that respiratory illness among adult civilians caused by adenovirus, as in the military cadre, is not a health problem of notable significance. Among the sporadic cases of frank clinical respiratory illness of adults studied, the proportion of cases caused by adenovirus has been less than 5% [29,31-34] and usually far less. In a few instances, intrafamilial spread of virus has been reported, and small-scale epidemics among adults in close contact with cases in children have been recorded.[24,26,27] On the basis of the present indication of low incidence of adenovirus-caused respiratory illness in adult civilians, general use of the vaccine in this group at this time is not justified and cannot be recommended.

The problem of adenovirus disease in children appears to be somewhat greater than in adults. Here also, however, the problem must be defined. Several sharp outbreaks of respiratory illness with conjunctivitis have been described among children in summer camps or schools where relatively large numbers of persons were brought together. Such outbreaks have been explosive in nature, with attack rates up to 60% in the children and 10% in the adults who were present.[11,27] Type 3 virus has been implicated in nearly all instances. Among children in family groups, adenovirus-caused respiratory disease appears more sporadic, and the attack rates are low.[33,34] The viruses recovered in such cases may be Types 1, 2, 3, 5, or 6. The problem of whether an adenovirus vaccine is needed for children, what virus types should be included in the vaccine, and, indeed, whether the vaccine would be effective in preventing disease, all remain important research problems for the future. At present, an exploration of possible usefulness of Type 3 adenovirus vaccine in a controlled study among children in summer camps and institutions is indicated. It should be remembered in any consideration of adenovirus vaccine that adenovirus disease is rarely, if ever, lethal. The disease is spontaneously regressive and poses neither a serious medical problem for the physician nor a significant economic burden upon the family.

Finally, in any consideration to employ adenovirus vaccine it should be noted that there is no evidence at present to relate adenovirus to the common cold. Adenovirus vaccine is not a cold vaccine. Indeed, in the controlled vaccine evaluation conducted at Fort Dix, N. J.,[17] during 1956, the bivalent adenovirus vaccine was essentially without effect in reducing the incidence of mild febrile (<100 F oral temperature) or afebrile cases which resembled the common cold clinically and in which the patients

were treated as outpatients at the dispensary.

Summary

Acute respiratory illness caused by Types 4, 7, or 3 adenovirus is a major problem in military medicine as it relates to the high hospital case attack rates in newly recruited soldiers during basic training. The disease is infrequent, by comparison, among seasoned men, and this is also true of the adult civilian population. The importance of adenoviruses in children's diseases remains to be determined definitively.

Vaccines effective in preventing at least 90% of the hospital cases of adenovirus-caused respiratory illness among new recruits have been developed and merit general use in military recruit populations. There is no indication for general employment of the vaccine at this time, either in "seasoned" military personnel or in the adult civilian population. The possible need for an adenovirus vaccine for children, the strain composition of the vaccine, and the assay of effectiveness of such a pediatric preparation remain important research problems for the future.

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Occurrence of Adenovirus Infections in Civilian Populations

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Acute respiratory infections constitute the commonest cause of illness,[1] yet progress in the identification of the viruses responsible for the great majority of these poorly differentiated infections has been slow and tedious. Isolation of the adenoviruses[2-4] represented the first significant contribution to the specific definition of the etiology of the common respiratory diseases since the discovery of the influenza viruses. The studies summarized by the papers of this symposium were all done during the last four years, and the scope of the data indicates how much has been achieved in this short period of time.

The adenoviruses comprise a group of agents possessing a common complement-fixing antigen, the group being separable into different types through the use of specific neutralizing antisera.[5] Types 4 and 7 have been related etiologically to an important respiratory disease of military recruits, acute respiratory disease (ARD),[2,6-8] a discovery which has permitted control of this disease with a vaccine containing these adenoviruses.[9,10] Since adenoviruses play such an important role in military populations, it was anticipated that these agents would be found to be responsible for a significant amount of respiratory disease in civilian groups, a circumstance which would prompt the use of adenovirus vaccine by civilians.

This paper reviews the data relative to the frequency of occurrence of adenovirus infections in civilian populations.

Infection with Type 3 Adenovirus

It is a curious fact that epidemics of respiratory disease due to Type 4 adenovirus have not been observed in civilian populations. Indeed, only three strains of Type 4 virus have been isolated from civilians, two by Evans[11] and one by Grayston.[12] An outbreak of illness due to Type 7 virus occurred in a boys' school in England,[13] but in general Type 7 infections have also been uncommon in civilian groups. This behavior of Types 4 and 7 adenoviruses is in marked contrast to the epidemic spread of these agents in military recruits. The closest counterpart in civilians has been the frequent epidemics of pharyngitis, with or without conjunctivitis, produced by the Type 3 adenovirus.[14-19]

Outbreaks of pharyngoconjunctival fever due to Type 3 virus have occurred in hospital patients,[14] families,[15,19] neighborhood groups,[16] and summer camps.[16,18] This virus also produces sporadic infections which may either resemble ARD[20] or be manifested only by conjunctivitis.[21] Unlike the current unique relationship between Type 4 and military recruits, Type 3 infections are not confined to civilians but also occur in military populations.[8,22]

Illnesses Caused by Other Types

The relationship of Types 4 and 7 to ARD and of Type 3 to pharyngoconjunctival fever is well established. Further, the

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epidemic occurrence of these illnesses makes clinical recognition easier and provides some measure of the frequency of infection. Such is generally not true of the other types. Although, as subsequently noted, antibodies to Types 1, 2, 5, and 6 are present in the sera of persons throughout the world and are commonly present as latent agents in the lymphoid tissue of the nasopharynx,[2,5] relatively few clinical illnesses attributable to these types have been documented.

Types 1, 2, and 5 have been isolated from children and adults with nonbacterial pharyngitis,[5,15,20] the manifestations being similar to those seen with Type 3 infections. How frequently infection with these types results in subclinical rather than overt illness is unknown and is a question that must be answered before the desirability of providing active protection against these agents can be evaluated fully.

Type 6 adenovirus has been isolated from patients with follicular conjunctivitis,[21,23] and Type 8, from cases of epidemic keratoconjunctivitis.[24] A constant association of keratoconjunctivitis and antibodies to Type 8 adenovirus has been demonstrated in sera from patients in a number of different countries,[25] providing additional evidence for the etiologic relationship between virus and disease.

A number of additional types have been discovered, and undoubtedly some of these will be shown to produce illness in man. This paper is limited to a consideration of Types 1 through 7. What percentage of the population has been infected with these agents? How often do they produce illness? What proportion of virus respiratory infections is due to adenoviruses?

The Presence of Antibodies

A similar complement-fixing antibody response presumably is provoked by infection with any of the adenoviruses. Thus, within the limitations imposed by the fall-off of such antibodies, assay of complement-fixing antibodies provides a measure of the

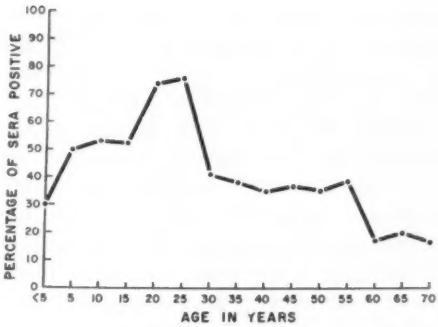

Fig. 1.—Age-frequency distribution of adenovirus complement-fixing antibody (data from Hilleman, Werner, Dascomb, and Butler[7]).

population's total experience with adenoviruses. Figure 1 is reproduced from the data of Hilleman et al.[7] and indicates that 50% of children have been infected with at least one type by the age of 5 years. The peak in the 20- to 25-year age group reflects the origin of the sera, for this group consisted mainly of soldiers only a few months beyond their basic training experience, when ARD and resultant antibody acquisition is so frequent. The percentage of positive sera then declines to approximately 20% in the 60- to 70-year-old persons. In England, Balducci, Zaiman, and Tyrrell[26] found complement-fixing antibody mainly in persons under 30 years of age.

The neutralization test permits measurement of the prevalence of type-specific

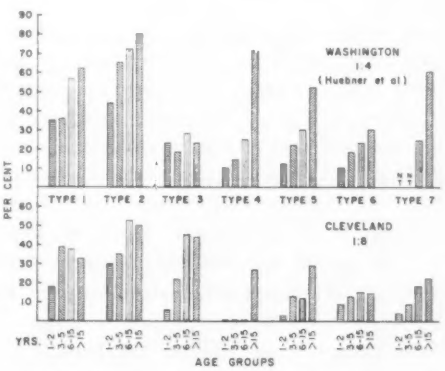

Fig. 2.—Type-specific neutralizing antibodies for adenoviruses in different age groups (Data relative to Washington[6] supplemented by Huebner and Rowe[27]).

antibodies. Figure 2 compares the results obtained in Washington, D. C., with use of a 1:4 dilution of serum [5] for screening with those obtained in Cleveland with a 1:8 screening dilution.[20] Sufficient data are not yet available to exclude the possibility that the lower dilution measures heterotypic antibody and nonspecific inhibitors. The apparent greater prevalence of antibodies in Washington is thought to be chiefly attributable to differences in the test procedures.

In general the distribution pattern of type-specific antibodies in a population of Cleveland families [1,20] is similar to that reported for indigent persons in Washington.[5] Antibodies to Types 1 and 2 adenoviruses are common in both groups. Despite the dilution difference, Type 3 antibody is more prevalent in Cleveland. Results in both groups confirm the curious inactivity of Type 4 virus in civilians. Huebner et al.[5] have interpreted the sharp rise in frequency of antibodies to Type 4, from 25% in the group 6 to 15 years of age to 71% in the group over 15, as indicative of the absence of epidemic Type 4 infections in Washington in the past 15 years. In the Cleveland population, Type 4 antibody was not found in children up to the age of 18, suggesting that this civilian group also has had little contact with Type 4 virus in recent years. In England, antibodies for all of Types 1 through 6 have been demonstrated in serum pools.[26]

With the exception of Type 6 in Cleveland, the prevalence of antibodies for the other types increases with age. In contrast to Type 4, Type 7 antibody is present in the younger age groups in Cleveland, suggesting that Type 7 adenovirus infections have not been entirely confined to military populations in recent years. However, Type 7 virus has not been isolated in either Cleveland or Washington.[27] Infections due to a variant, tentatively identified as Type 7′, have occurred in civilian populations in Canada and England, and one strain of this virus has been isolated in the Washington area.[27]

Thus, serologic studies indicated that many persons have been infected with adenoviruses. Infections continue to occur throughout adolescence, and there is a gradual increase in the number of type-specific antibodies with age, so that virtually all adults have antibodies to at least one type.[5,20] That many of these infections are accompanied by clinical illness, as in the case of Type 3 virus, has been well documented. How many infections occur without clinical symptoms, particularly in infancy and childhood, is the subject of current studies by investigators in Cleveland, Chicago, Kansas City, and Washington. It is not yet known whether each antibody acquisition is accompanied by a respiratory illness, but data are available regarding the frequency with which adenoviruses produce respiratory disease in civilian populations.

The Frequency of Infection

The experience of investigators who have studied respiratory illnesses in civilian groups is summarized in Table 1. When sera from hospitalized patients with illnesses most closely resembling adenovirus infections clinically were selected for testing, 18% of the patients showed increases in titer of neutralizing antibody.[20] However, when there was no such selection, only 0.5% to 3.0% of patients hospitalized with respiratory symptoms showed evidence of adenovirus infection.[11-13,28] Virus isolation attempts have resulted in low yields in all groups studied. Adenoviruses were isolated from only 2% of respiratory illness suffered by persons in a family population in Cleveland [20] and from 0.5% to 2% of illness in other groups of adults and children.[11,12] Although college students are comparable in age to military recruits, only 0.5% to at most 3% of their illnesses are related to the adenoviruses.[11,12,28] Jackson [30] found serologic evidence of adenovirus infection in less than 3% of adults reporting to a university health service with acute respiratory infections.

TABLE 1.—*The Frequency of Simultaneous Adenovirus Infection in Persons with Acute Respiratory Illnesses*

Author & Location	Population Studied	Cases, No.	Methods of Diagnosis	+ Adenovirus	
				No.	%
Jordan et al. [20] Cleveland	Selected hospital patients	68	Neutralization	12	18.0
	Families	706	Virus isolation	15	2.1
Grayston [15]	Hospitalized adults	150	Virus isolation	1	0.7
	College students	390	Virus isolation	2	0.5
Chicago	Infants & children *	627	Virus isolation	8	1.3
Evans [11] Madison	Hospitalized college students	290	Virus isolation	5	1.7
		225	Complement-fixation	6	2.7
Tyrrell et al. [12]	Adults hospitalized with bronchitis and pneumonia	66	Complement-fixation	2	3.0
England	Students & nurses	40	Complement-fixation	0	0
Minuse & Davenport [28] Ann Arbor	Hospitalized college students (1943–1956)	814	Complement-fixation	24	2.9
Grist et al. [29] Glasgow	Children & adults in hospital and practice	205	Complement-fixation	1	0.5
Jackson [30] Chicago	University employees	124	Complement-fixation	3	2.4

* The majority, but not all, of these had respiratory illnesses.

Table 2 summarizes the results obtained by several investigators when paired serum specimens collected before and after winter respiratory-disease seasons were examined for evidence of adenovirus infection during the intervening months. When the neutralization test was used, the percentage of persons showing increases in titer varied according to the virus type. Considering all such rises as meaningful, it was calculated that 4% of the illnesses which occurred in a group of Cleveland families could be attributed to infection with adenoviruses.[20] This estimate agrees closely with the percentage figures obtained by Price,[31] using the complement-fixation test. The frequency of infection in college students measured by widely spaced serum specimens [32] was similar to that detected by studying individual illnesses in such students (Table 1).

Comment

In marked contrast to their behavior in recruit populations, the adenoviruses have been related etiologically to but a fraction of the respiratory illnesses experienced by a number of different segments of the civilian population. While the efficacy and desirability of administering adenovirus vaccine to military recruits has been established, the evidence cited indicates that such immunization of civilian populations, including university students and older adults, is not warranted.

Serologic data, for both neutralizing [5,20] and complement-fixing [7] antibodies, indicate that 50% of children have been infected with at least one type of adenovirus by the age of 5 years. To prevent these infections, particularly the household epidemics of pharyngoconjunctival fever, it can be argued

TABLE 2.—*Serologic Evidence of Adenovirus Infection in Persons During Winter Months*

Author & Location	Population Studied	Persons, No.	Method	% with Increase in Titer
Jordan et al. [20] Cleveland	Adults	120	Neutralization (7 types)	0–1.7 *
	Children	138		0–6.8
Price [31] Baltimore & Maryland	College students †	2015		4.0
	Adults in clinic	1051	Complement-fixation	4.0
	Adults in state	1115		4.3
Katz [33] Cleveland	College freshmen	102	Complement-fixation	2.8

* Per cent varied with type.
† Bled every 4 months over a 2-year period.

that adenovirus vaccine should be given in infancy. However, adenovirus illnesses are so outnumbered by the common cold and other virus respiratory infections that such immunization would have little effect on the total respiratory disease rate. With data provided by the Cleveland family study,[33] it can be calculated that the average child has had 49 common respiratory diseases by the end of the first five years of life. No more than two of these illnesses can be attributed to adenoviruses on the basis of present evidence. By the end of the 10th year, the average child has had 78 common respiratory diseases; even if five of these were related to adenoviruses, a 100% effective vaccine would reduce the number of respiratory illnesses suffered by the child by only 6%. Such a small return does not justify the investment of time and money which would be necessary for the widespread immunization of children.

Summary

The data regarding the occurrence of adenovirus infection in civilian populations are summarized and reviewed. In marked contrast to their prevalence in military recruits, infections due to Type 4 and Type 7 adenoviruses, particularly Type 4, have been and are infrequent in civilians. Type 3 virus has most often been responsible for epidemics in civilian groups.

Serologic studies indicate that many persons have been infected with adenoviruses. By the age of 5 years, 50% of children have been infected with at least one type. Infections continue to occur throughout adolescence, and nearly all adults have antibody to at least one type of virus. During a given respiratory season, however, only approximately 4% of the civilian population is infected with an adenovirus, and these agents account for but from 0.5% to 3% of the respiratory illnesses.

It is estimated that use of a completely effective adenovirus vaccine would result in only a 6% reduction in the number of common respiratory illnesses experienced by an average child during the first 10 years of life. Present evidence does not warrant adenovirus immunization of such civilian groups as families or university student populations.

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The Special Problem of Rheumatic Heart Disease in Pregnant Women

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Mr. Chairman, let me express my gratitude for the privilege of delivering this lecture. Dr. Walter L. Bierring, present here today, was good enough to dip down into the inexhaustible well of his historical knowledge and tell me something about the founding and development of this lectureship. It was proposed in 1927 by Dr. Henry A. Christian. The original five trustees were Lewellys F. Barker, Rollin T. Woodyatt, Eugene F. Kilgore, Warfield T. Longcope, and Walter L. Bierring. In 1928 the Section voted to name the annual lecture in honor of Dr. Frank Billings of Chicago. This was in recognition of Dr. Billings' many important contributions to the activities of the Section and of his general eminence as a teacher, practitioner, and statesman of medicine.

The Billings Lecture, in my view, has three distinctions. It is named for a great figure in American medicine, it is the official lectureship of the Section on Internal Medicine, and the 25 previous lecturers make up a list to admire.

The lecture today will deal with the special problem of rheumatic heart disease in pregnant women. Before this problem is presented in specific terms certain general observations may be made. It is a fundamental tenet of our political belief that all men are created equal and Mr. Robert Burns wrote, "A man's a man for a' that." These observations are not to be taken as implying that all members of the human race are identical. They are indeed different. Not only is one individual person different from another, but a given person is not physiologically the same at all times. The varying characteristics of different persons and the changes in persons at different times can affect the incidence and the course of disease.

It is generally accepted that men are different from women and that this difference affects the incidence and course of disease in these two groups. Some diseases are sex-limited; many are sex-influenced. When Prof. John Morse was teaching pediatrics to students in the Harvard Medical School he was accustomed to say, "A baby is not a little man." He meant that a baby was qualitatively, as well as quantitatively, different from an adult—that is, that physiological regulation is different in babies from physiological regulation in adults and that it is necessary to know something about this difference to recognize and manage the baby's disease.

There is a difference in the physiology of a given person between waking and sleeping. Students of homeostasis have long been impressed with the stability of the mechanism controlling the level of carbon dioxide tension in the arterial blood. And yet, as recently shown by Robin and his co-workers,[1] the onset of sleep is accompanied by a sudden alteration in the sensitivity of the respiratory center to car-

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bon dioxide stimulation, and a new chemical equilibrium is established with carbon dioxide tension at a measurably higher level than in the waking state.

Pregnancy is a striking example of this general principle that people are physiologically different at different times. It is generally accepted that a pregnant woman is in many ways a different organism from her nonpregnant self, and pregnancy is well known to influence the incidence and the course of many diseases. For many years, beginning in 1932, in the Department of Medicine at Vanderbilt University, my colleagues and I have been trying to define some of the physiological changes which accompany pregnancy.[2,3] Since 1950 Dr. James Metcalfe and I have had the privilege of operating a research laboratory and an active medical service at the Boston Lying-in Hospital—an opportunity which we hold because of Prof. Duncan Reid. Here we have been able to continue a systematic investigation of some of the alterations in the circulation and respiration of pregnant women, and at the same time we have been able to observe and to study a substantial number of patients with rheumatic heart disease as they go through pregnancy, delivery, and the postpartum period.

During the six-year period we have encountered and followed 277 pregnant women in whom a firm diagnosis of cardiac disease could be made. Table 1 shows the classification of these patients according to the major etiological factor in the production of the heart disease. This table demonstrates—not for the first time—that rheumatic heart disease makes up the vast majority of cardiac disorders encountered in women of the child-bearing age. In this group of pregnant women 85% of the heart disease was rheumatic. Almost all the rest represented congenital deformities.

TABLE 1.—*Etiological Classification of Heart Disease in Pregnant Women*

Type of Heart Disease	Patients		Pregnancies	
	No.	%	No.	%
Rheumatic	236	85	298	84
Congenital	35	12	49	14
Hypertensive	2	1	3	1
Other	4	2	5	1
	277	100	355	100

The predominant valve lesion in each patient with rheumatic heart disease has been identified, and in approximately two-thirds of these pregnancies the predominant lesion was mitral stenosis. It is fair to say, therefore, that the problem of heart disease in pregnancy is usually rheumatic heart disease, and in about two-thirds of all patients the major difficulty is mitral stenosis.

It has already been emphasized that to understand disease processes in any person it is necessary to consider not only the mechanisms of disease but the physiology of the individual person. We turn now to a discussion of some of the changes in physiology associated with pregnancy which may influence the manifestations and the management of heart disease. Because mitral stenosis is the most frequent cardiac disorder encountered in pregnant women we shall emphasize those changes in the circulation which may create special problems for the patient with mitral stenosis.

It is a commonplace to say that pregnancy increases the metabolic requirements. One measure of metabolic requirements is the total oxygen consumption of the mother. This has been studied in pregnant women by many groups of workers, including ourselves. The total oxygen consumption begins to rise measurably toward the end of the first trimester. It rises steadily throughout pregnancy and reaches its maximum at term. This maximum is usually 15% to 18% above the nonpregnant level for the person. This does not sound like a very demanding metabolic burden. However, the total oxygen consumption is only an indirect indicator of the burden borne by the heart and circulation, and it would be better to make direct observations on the circulation itself. Consider first the simple matter of the cardiac rate. When this is studied under careful basal conditions repeatedly and in a number of patients and charted according

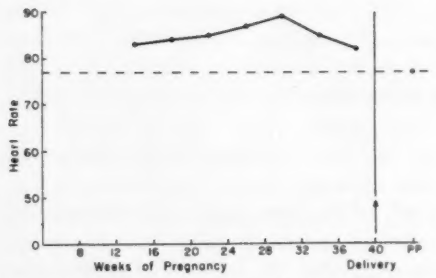

Fig. 1.—Heart rate during and after pregnancy.

to the months of pregnancy, a curve is obtained which is shown in Figure 1. It is seen that the heart rate rises early in pregnancy—earlier than the oxygen consumption; that it reaches a maximum between the 30th and 34th weeks, and that it is lower in the last weeks of pregnancy than at this maximum. The usual increase amounts at most to 10 or 12 beats per minute. This may not sound impressive but it adds up to something like 15,000 extra beats per day. Moreover, the rise in heart rate in response to exercise is greater in pregnant women than in the same women when they are not pregnant.

The arterial blood pressure has been measured under similarly standard conditions. The systolic pressure does not alter significantly; the diastolic pressure falls and reaches its lowest point in the eighth or ninth month, rising somewhat before delivery.

A measure more significant for our present problem is that of the actual output of blood by the heart. This has been studied by many methods in many laboratories. There is now general agreement as to what happens. The cardiac output rises, and it rises out of proportion to the oxygen consumption, so that whereas the maximum increase in oxygen consumption is about 15% to 18%, the maximum increase in cardiac output is often 40% to 50%. Moreover, the maximum cardiac output does not come at the peak of oxygen consumption but some weeks before this, so that the curve of cardiac output during pregnancy is of the general shape shown in Figure 2. The cardiac output is less in the last weeks of pregnancy than at the maximum.

The total blood volume follows a curve of generally similar shape. The increase in blood volume is due partly to an increase in red cell mass and partly to an increase in plasma volume.[4] The plasma volume increases relatively more than the red cell mass, so that at the point of maximum blood volume some dilution of the hemoglobin is present. There is an interesting difference in the time relation between the changes in red cell mass and of plasma volume. The curve of the red cell mass goes steadily upward until term, as does the curve of total oxygen consumption. The curve of plasma volume turns down at the seventh or eighth month and is lower in the last weeks of pregnancy than at its maximum. This diminution in plasma volume occurs in spite of a continuing and steady climb in total body water, so that it is to be taken as a distribution phenomenon and not merely as fluid retention.

While these striking and fascinating changes in the circulation are going on, certain relevant changes are also taking place in the respiratory mechanism. Those to be mentioned include the vital capacity, the ventilation per minute, and the level of carbon dioxide pressure in arterial blood. Any sensible person looking at a pregnant woman and uninformed about the truth of the matter would conclude that her vital capacity was diminished. By a generous provision of nature, however, this is not

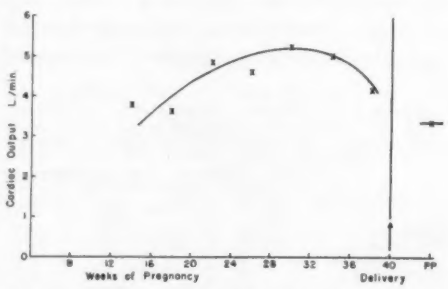

Fig. 2.—Cardiac output during and after pregnancy.

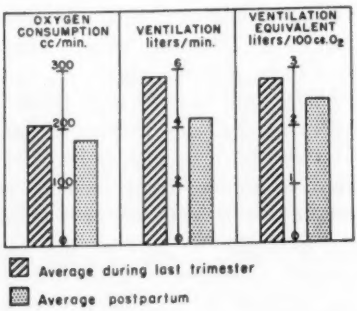

Fig. 3.—The hyperventilation of pregnancy.

the fact, and in normal women the vital capacity is not diminished during pregnancy but on the contrary is somewhat increased. This is not a new observation. It was well known to Angus MacDonald,[5] when in 1878 he wrote the first book in the English language on heart disease in pregnancy, and it has been rediscovered periodically since then.

A second and equally interesting observation is that of the change in total ventilation. The total ventilation, like the total cardiac output, increases out of proportion to the oxygen consumption. While the oxygen consumption goes up 15% to 18%, the total ventilation per minute goes up about 40%, as is shown in Figure 3. That this is true hyperventilation is shown by the fact that pregnancy is accompanied by a fall in arterial carbon dioxide tension, usually by about 10 mm. of mercury. We do not have time to discuss the interesting mechanisms which may be involved in this change, but it has one good result. Considering the probable gradient of carbon dioxide across the placenta, this hyperventilation means that the fetus can develop at an essentially normal carbon dioxide tension.

Now let us consider the application of these observations to the management of heart disease in patients. Since mitral stenosis is the most frequent cardiac problem observed in pregnant women, let us consider specifically how these changes of the circulation and respiration during pregnancy can adversely affect a patient with mitral stenosis. I remind you, first, that an increase in the cardiac output of a patient with mitral stenosis can be achieved only by an increase in the pressure gradient across the mitral valve, that is, by an increase in the left atrial pressure. Such an increase in pressure leads inevitably to a rise in pressure in the pulmonary veins and in the pulmonary capillaries.

Second, an accelerated heart rate is known to be a factor which may precipitate pulmonary congestion in patients with mitral stenosis. An increase in heart rate leads to diminution of available diastolic time, that is, to a diminution of the time available for blood flow through the mitral valve. Thus, when the rate increases, if there is to be a maintenance of blood flow through the mitral valve, there must be a further rise in left atrial pressure.

Third, an increase in total blood volume can, through various mechanisms, lead to an increase in the pressure level in the left atrium. The changes in cardiac output, in heart rate, and in blood volume are to a considerable extent inseparable parts of pregnancy. They may be somewhat modified or limited. They can seldom be abolished and probably ought not to be abolished. They must, therefore, be understood and in part accepted. They mean that the pregnant woman with mitral stenosis, because she is pregnant, has a higher left atrial pressure. Moreover, this elevated left atrial pressure exists in a woman who because she is pregnant has an elevated ventilation volume and is, therefore, that much nearer to dyspnea.

Knowing these facts about the changes which pregnancy induces in the maternal organism and knowing something about the dangers that these changes may present to the woman with heart disease, there are three varieties of approach to the question of management: 1. The problem may be avoided by terminating the pregnancy. This is not a good solution. The fetal mortality is 100%. There is some risk to the mother even early in pregnancy. Psychologically the effects are generally bad, and the pro-

cedure is often quite unacceptable to the patient. Happily, as our own experience of heart disease has accumulated, the number and per cent of interruptions have diminished, and this procedure is now considered as only rarely indicated. In the last 18 months of the period of our experience under scrutiny, interruption was advised only once. 2. Since the problem of mitral stenosis is essentially a mechanical one, the suggestion is frequently made that a good principle of management is to modify the mitral stenosis by surgery during pregnancy. We believe that this also is not a good solution, partly because our experience has shown that it is almost never necessary and partly because we are aware of certain special risks of cardiac surgery in pregnant women. There may well be rare and special situations which indicate the use of cardiac surgery during pregnancy, but these have not been encountered in our experience and will seldom be encountered in a well-organized medical clinic. Our policy is to postpone cardiac surgery until pregnancy has been completed. 3. The third method of management is not dramatic, but it is highly sensible and highly successful. It is the method of conservative medical management.

According to our experience the ability of a patient with heart disease to go successfully through the dangers of pregnancy, delivery, and the puerperium depends on two factors: (1) the capacity of the heart for work and (2) the extent of the total demand for work which is placed upon the heart during pregnancy.

It is necessary to have a clear concept of what is meant by the total demand on the circulation. This total demand includes the cardiac work necessary to sustain life and the cardiac load imposed by usual activity, by emotional stress, by illness, by overweight, by lack of sleep, and by many other factors. Pregnancy is only one segment of the total load. In the famous case of the camel's back it was the total load that was important. The last straw was significant only because it brought the total burden to the critical and breaking point. The first principle, then, of managing the combination of pregnancy and heart disease is to make a place in the patient's cardiac budget for the expenditures of pregnancy by removing enough other burdens from the total load to compensate for the burden of pregnancy.

Some examples of burdens that are to some degree avoidable or removable are as follows: physical activity, emotional stress, ectopic rhythms with tachycardia, anemia, obesity, infections, hyperthyroidism, infusions or transfusions, and variations in sodium intake or retention. Most of the activity of the doctor in caring for pregnant women with heart disease has to do with the control of such factors. It is true that the physician can to some degree minimize the burden of pregnancy itself. Cornett,[6] in our laboratory at the Boston Lying-in Hospital, has shown that the increase in total blood volume can be minimized by a low-sodium diet. But it cannot be too clearly stated that the specific burdens of pregnancy cannot be abolished. It can also be said, on the basis of our experience, that under good management these burdens can be safely endured by almost every woman with heart disease. The management of heart disease in pregnant women is seldom a problem of the use of digitalis or of diuretics. It is a matter of the prevention and control of congestive phenomena by understanding and controlling the total burden on the heart.

A test of the validity of the concepts that have been presented in this lecture is the recorded experience of heart disease as managed by various methods. Angus MacDonald reported his experience in 1878. He saw a relatively small group of patients with severe heart disease who came to him late in pregnancy and after the development of heart failure. Dr. MacDonald's results in terms of maternal mortality are shown in Table 2. This terrifying summary represents the kind of experience that led to the growing up of that kind of medical

TABLE 2.—*Mortality from Heart Disease in Pregnancy in 1878*

Predominant Valve Lesion	Patients, No.	Deaths, No.	Maternal Mortality, %
Mitral stenosis	14	9	64
Mitral regurgitation	8	4	50
Aortic regurgitation	6	3	50
Others	3	3	100
Total	31	19	61 (av.)

TABLE 3.—*Mortality from Heart Disease in Pregnancy in the Present Series*

Functional Class	Pregnancies, No.	Maternal Deaths, No.
I	164	0
II	62	0
III	24	1
IV	48	2
Total	298	3

folklore that says that women with heart disease cannot safely have babies.

More than 30 years ago Dr. Burton Hamilton began his long and productive study of the care of heart disease in pregnant women. Figure 4, taken from figures in the book by Hamilton and Thomson,[7] records the fall in maternal mortality which resulted from his wise and careful application of the principles that have been referred to here.

Our own experience as to maternal mortality is summarized in Table 3. This represents the 298 pregnancies through which patients with rheumatic heart disease have gone under our supervision. In this series there have been three deaths. One patient with aortic stenosis died suddenly after the interruption of pregnancy, one patient with a twin pregnancy died some weeks after mitral valve surgery (which had not been advised by us), and one patient with aortic disease died of Staphylococcus pneumonia. On the basis of this experience we conclude that in terms of immediate maternal mortality the results of conservative management on these principles are good.

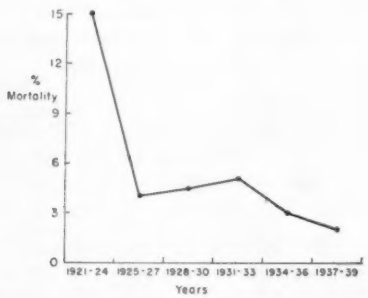

Fig. 4.—Maternal mortality in the series of Hamilton and Thomson.[7]

The next question has to do with the long-term results for mothers. Does the experience of pregnancy in a woman with heart disease make the heart disease worse even if she survives the pregnancy? This question has been studied in our laboratory by Miller and Metcalfe.[8] They reviewed our first 100 patients 4.1 years, on the average, after they were seen during pregnancy. Four years after pregnancy only a few of these women were worse than during pregnancy. Most of them were in the same functional class in which they had been observed originally, and 27, a surprising number of them, were better. Only 5 of the 27 who were classed as better had had cardiac surgery—4 for mitral stenosis and 1 for coarctation. In this experience there is no evidence that pregnancy once survived had accelerated the course of heart disease.

So much for the maternal results. Now what about the results in terms of babies? The answer to this we shall illustrate by recording the immediate fetal and neonatal mortality in our series, and relating it to the severity of the heart disease in the mothers. It is seen in Table 4 that in Group I the combined fetal and neonatal mortality is 7%—a figure comparable to that obtaining in women without heart disease. It is also clear from these figures that as the maternal heart disease is severer the fetal mortality is higher. Some of this total fetal mortality has been due to the fact of interruption of pregnancy. Most of this kind of fetal mortality has been eliminated in recent years, but it is quite apparent that at this stage of our knowledge fetal mortality will be high in Group IV patients even if the pregnancy is permitted to con-

TABLE 4.—*Fetal Mortality Related to Severity of Maternal Heart Disease*

Maternal Functional Class	Pregnancies, No.	Fetal and Neonatal Deaths		
		Spontaneous, No.	Interruptions, No.	Total %
I	164	10	2	7
II	62	5	3	13
III	24	2	7	37
IV	48	10	16	54
Total	298	27	28	18 (av.)

tinue. This fact must be taken into consideration in planning the management of women in Group IV.

Conclusion

This total experience indicates to us that in the vast majority of instances it is possible to carry women with heart disease safely and productively through pregnancy. Success in this endeavor depends on the understanding and careful application of the principle of the total cardiac burden and on making a wide appraisal of the factors, in pregnancy and in other aspects of the patient's life, which influence this burden. The success with which the principle can be applied depends, among other things, on the cooperation of the patient, the persuasiveness of the doctor, and the availability of appropriate services in the community. When the principle can be applied to the problem of heart disease in pregnancy the results in general are good.

Pregnancy is an example of the principle that changes in the host can and do influence the course of disease. It is a special example because the changes of pregnancy are relatively standardized and because they are reversible. Pregnancies come to an end, and this permits improvement in cardiac symptoms and a comparison of the pregnant organism with the same organism in the nonpregnant state.

The lessons that have been learned from our experience with pregnant women can be directly and helpfully applied to the understanding and the management of nonpregnant patients with heart disease.

In the first edition of "The Principles and Practice of Medicine," by William Osler, there is a quotation from Plato which is relevant to the principle underlying this lecture. This reads as follows: "And I said of medicine, that this is an art which considers the constitution of the patient, and has principles of action and reasons in each case."

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Further Clinical and Investigative Uses of Liver Biopsy

An Analysis of Five Hundred Twenty-Seven Biopsies

K. L. STUART, M.D.; G. BRAS, M.D.; S. J. PATRICK, Ph.D., and J. C. WATERLOW, M.D., Jamaica, B. W. I.

It is no longer a matter of controversy whether needle biopsy of the liver is a justifiable procedure in clinical medicine. Most authorities agree that the advantages of its discriminative use outweigh its dangers, and in experienced hands with proper precautions the risk to the patient is almost negligible. The number of excellent reports on liver biopsy that have appeared in recent years, particularly those by Sherlock,[1] Gillman and Gillman,[2] Hoffbauer,[3] Schiff,[4] and Popper and co-workers,[5] underline the increasing importance of this procedure and its contribution to the study of liver disease. It has so far had its widest application clinically and has become one of the main diagnostic tools in the differential diagnosis of a wide range of liver diseases. Zamcheck and Sidman [6] have recently listed a number of other diseases not primarly hepatic in origin in which liver biopsy has been usefully employed. These include such varied conditions as amyloidosis; Wilson's disease; hemochromatosis; syphilitic cirrhosis; cardiac cirrhosis; granulomatous diseases, such as leprosy, brucellosis, sarcoid, and fungus infections; inflammatory diseases, such as infectious mononucleosis, Weil's disease, and toxic hepatitis from cincophen, chloroform, and arsenic poisoning; certain focal necroses, such as tularemia, syphilis, liver abscess (both bacterial and amebic), periarteritis nodosa, lupus erythematosus, leukemia, Hodgkins' disease, and a number of parasitic diseases, such as kala-azar and schistosomiasis. Its potentialities for basic histopathological and chemical research are also being increasingly recognized, and the indications for its use are now well defined.

With the opening of the University College Hospital of the West Indies, in September, 1952, it soon became evident that there were a number of conditions occurring locally in which a detailed knowledge of the histological and biochemical changes in the liver would be of value. Some of these, e. g., kwashiorkor, marasmus, and sickle-cell anemia, occur in other tropical countries and have been the subjects of many previous reports. Others, however, such as veno-occlusive disease of the liver and the vomiting sickness of Jamaica, had not been reported from other areas, and detailed clinical, histological, and biochemical studies of the associated hepatic changes had not been previously undertaken. In the study of these conditions we have used liver biopsy with particular advantage (1) as a clinical tool, especially when serially performed, in establishing the clinical diagnosis; in following the course of the disease, and in assessing prognosis and response to treatment and

TABLE 1.—*Clinical Conditions and Patients Studied by Liver Biopsy*

Disease	Biopsies No.	Biopsies %	Patients No.	Patients %
Kwashiorkor	158	30.0	36	23.6
Marasmic kwashiorkor			24 (78)	
Marasmus			18	
Veno-occlusive disease	173	32.8	84	25.4
Vomiting sickness	29	5.5	16	4.8
Diabetes mellitus	36	6.83	24	7.3
Neuropathies	19	3.6	19	5.7
Nephrosis	10	1.9	10	3.0
Sickle-cell anemia	21	4.0	18	5.4
Unexplained hepatomegaly	47	8.9	47	14.2
Unexplained splenomegaly	10	1.9	10	3.0
Unexplained jaundice	16	3.0	16	4.8
Unexplained pyrexia	5	1.0	5	1.5
Unexplained anemia	3	6.6	3	0.9
Total	527		330	

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From the Departments of Medicine, Pathology, and Biochemistry of the University College of the West Indies and the Tropical Metabolism Research Unit of the Medical Research Council.

TABLE 2.—*Clinical Summary, Liver Function Tests, and Liver Biopsy Findings in Ten Characteristic Cases of Veno-Occlusive Disease*

Case No.	Clinical Type (on Admission)	Time of Test	Liver Function Tests							Liver Biopsy (or Postmortem) Findings*	Result
			Bilirubin (1 Min.), Mg./100 Ml.	Bilirubin (Total), Mg./100 Ml.	Thymol Turbidity Units	Cholinesterase, Units	Total Protein, Gm./100 Ml.	Albumin, Gm./100 Ml.	Globulin, Gm./100 Ml.		
1	Acute	Admission	0.1	0.7	2.3	0.3	5.0	2.5	2.5	Acute V. O. D.	
		After 4 ½ wk. (clinical recovery)	0.2	0.7	2.8	0.8	7.8	4.1	3.7	Normal	Full recovery
2	Acute	Admission	--	1.0	3.4	0.3	5.3	3.5	1.8	Acute V. O. D.	
		After 4 wk. (improved)	0.1	0.8	1.6	0.47	7.0	4.6	2.4	Acute V. O. D.	Full recovery
		After 8 wk. (recovered)	--	0.6	2.0	0.65	8.1	4.5	3.6	Normal	
3	Acute	No symptoms (routine)	0.1	0.5	2.8	0.61	8.5	4.3	4.2	----	
		Onset of illness (admission)	1.06	2.0	1.6	0.36	5.8	2.9	2.9	Acute V. O. D.	Full recovery
		After 4 wk. (very ill)	2.6	4.0	2.1	0.13	5.6	2.5	3.1	----	
		After 8 wk. (recovery)	1.2	2.4	2.9	0.75	7.7	4.2	3.5	Normal	
4	Acute	Admission (very ill)	2.4	4.2	2.8	0.14	5.8	2.4	3.4	Severe acute V.O.D. (postmortem)	Death in hepatic failure 15 days after admission
5	Acute	First admission	0.3	2.0	1.6	0.21	4.8	2.7	2.1	Acute V. O. D.	
		After 8 wk. (v. ill)	0.4	2.0	5.0	0.09	4.9	1.9	3.0	V.O.D. with centrilobular fibrosis	
		After 3 mo. on discharge	--	--	3.0	0.40	6.9	2.8	4.1	Marked centrilobular fibrosis	Steady progression to cirrhosis and death from hematemesis 15 mo. from acute onset
		Second admission (6 mo. assessment)	--	--	1.4	0.58	6.3	4.3	2.0	Nonportal cirrhosis	
		Third admission (hematemesis)	0.4	1.6	3.3	0.27	6.8	2.8	4.0	Nonportal cirrhosis (postmortem)	
6	Acute	Admission	2.2	6.0	2.7	0.39	5.1	2.2	2.9	Acute V. O. D. (postmortem)	Death in hepatic failure 10 days after admission
7	Subacute	First admission	--	--	2.8	0.31	6.1	3.0	3.1	V.O.D. (typical but not marked)	
		After 6 wk. (on discharge)	0.1	0.6	3.8	0.47	7.6	4.5	3.1	Progressive fibrosis	Clinical improvement but with progressive hepatic fibrosis
		Second admission	0.2	0.9	4.2	0.50	7.5	4.0	3.5	Further fibrosis	
		After 3 wk. (on discharge)	--	0.8	4.3	0.46	7.3	3.6	3.7	Further fibrosis	
8	Subacute	Admission (symptomless hepatomegaly)	0.1	1.1	1.0	0.4	7.7	2.4	5.3	V.O.D. (typical but not marked); repeat biopsy not obtained	Clinical improvement
9	Chronic	Admission	--	0.8	4.0	0.37	8.4	3.9	4.5	Liver biopsy and postmortem findings; chronic V.O.D. with cirrhosis	Death from hematemesis 11 wk. after admission
10	Chronic	Admission	--	--	2.4	0.35	7.8	3.0	4.8	Liver biopsy and postmortem findings —chronic V. O. D. with cirrhosis	Death from hematemesis 17 days after admission

* V.O.D. indicates veno-occlusive disease.

(2) as an investigative procedure in determining the type, duration, and severity of the pathological and biochemical changes in a number of cases in which the clinical diagnosis was not in doubt. In this paper are given (1) a clinical outline of some of the less widely known diseases which we have studied by liver biopsy — veno-occlusive disease of the liver, kwashiorkor, marasmus, and the condition which has been known in Jamaica for many years as "vomiting sickness"; (2) the histopathological and biochemical information obtained from liver biopsy in these conditions and its unique contribution to our present understanding of them; (3) the advantages of simultaneous histopathological and chemical examination of portions of the tissue obtained at biopsy; (4) our experience with liver biopsy in some of the more familiar clinical circumstances, such as unexplained hepatomegaly, splenomegaly, and jaundice. The liver-biopsy findings in sickle-cell anemia; nephrosis; diabetes; a neuropathic syndrome, probably nutritional, recently reported from Jamaica, and other miscellaneous conditions are also described.

Veno-Occlusive Disease of the Liver

The clinical and pathological features of this syndrome in children and in adults in the West Indies have been given in previous papers.[7-10] Although most reports of the disease have been from Jamaica, its occurrence in Barbados has also been reported.[11] It occurs particularly in inadequately nourished children and has been described as having three clinically recognizable stages. The acute stage (Cases 1 to 6, Table 2) is the most dramatic and is characterized by the abrupt onset of hepatomegaly and ascites. The enlargement of the liver is often marked, frequently extending 6 to 8 cm. below the umbilicus. In the majority of cases repeated reaccumulations of ascitic fluid occur, and numerous paracenteses may be necessary. Complete recovery may occur clinically, histologically, and on laboratory examination, or death may ensue in hepatic failure. There is an intermediate subacute stage (Cases 7 and 8, Table 2) of usually symptomless hepatomegaly, which is often discovered for the first time on routine examination or on examination for an unassociated complaint. Alternatively, direct progression from the acute to the subacute stage may be observed. A number progress either clinically or subclinically to the final stage (Cases 9 and 10, Table 2), which is characterized by a chronic fibrosis, congestive splenomegaly, and recurrent hematemeses. Jaundice sometimes occurs in this or earlier stages of the disease and is of serious prognostic significance. Some patients may be seen for the first time in the subacute or chronic stage of the disease, with no preceding history of the acute stage. In such cases the differential diagnosis from other causes of hepatomegaly or other types of cirrhosis may be difficult in the absence of liver biopsy. The essential histological lesion in the initial stages is centrilobular necrosis and congestion with occlusion of the smaller and medium-sized branches of the hepatic veins. In the later stages there is a progressive nonportal fibrosis.

Veno-occlusive disease of the liver has been shown to be the commonest cause of hepatic cirrhosis in children in Jamaica, and it is also responsible for a large proportion of cases of adult cirrhosis.[9] The precise etiology of the disease is unkown, and the role of undernutrition, undetermined. Malnutrition of some degree, however, is invariably present and is thought to play an important but subsidiary role, probably as a contributory cause and in affecting the prognosis. The probably more specific role of a plant toxin has been already discussed, and the evidence incriminating species of Crotalaria and Senecio taken locally in the form of "bush teas" has been recently presented.

Kwashiorkor and Marasmus

Kwashiorkor, as seen in Jamaica, presents a very variable picture and usually does not conform to the complete syndrome initially

described in equatorial Africa by Williams [12] and later by other workers.[13,14] Waterlow has noted the relatively infrequent occurrence of the marked dermatoses usually seen in the African cases.[15] The typical examples of the syndrome in the present series tended to be well covered with blubbery fat and, although markedly edematous, were not desperately ill, responding very easily to treatment. The marasmic group presented the usual features of thin wasted atrophic muscles, often severe dehydration, and marked loss of subcutaneous fat. Depigmented hair with a fine silky texture, cheilosis, angular stomatitis, glossitis, anemia, and hepatomegaly were found in the majority of infants in the kwashiorkor group and in many with marasmus. Hypoproteinemia is a feature of each group but was more marked in patients with kwashiorkor (Table 5). In a recent report, Jelliffe, Bras, and Stuart described a clinically intermediate subgroup, which they termed marasmic kwashiorkor.[16] No distinct criteria, however, have been set up for this subgroup, and it has been merely used to denote a group of cases with rather ill-defined borders that lie somewhere between "classical" kwashiorkor on the one hand and marasmus on the other. In all cases in these three groups, as in the cases described from Africa by Gillman and Gillman [13] and by Trowell, Davies, and Dean,[14] there was nearly always a dietary history of a very low protein intake.

The Vomiting Sickness of Jamaica

Full clinical reports of this syndrome have been given elsewhere.[17,18] It is often a rapidly fatal illness, characterized by vomiting, drowsiness, convulsions and coma, severe hypoglycemia, and hepatic glycogen depletion. Children are primarily affected, especially between the ages of 1 and 10 years. The illness occurs either sporadically or in small family outbreaks and reaches its highest incidence between December and March. The relatively undernourished are again the most seriously affected. Two main clinical types are recognized: 1. The abrupt onset of vomiting without pain, fever, diarrhea, or previous malaise. There is rapid prostration, often followed by a period of remission. After a time the vomiting recurs and is followed by drowsiness, twitching of the limbs, convulsions, coma, and death. If recovery takes place, it is usually complete within 48 or 72 hours, and there are no after-effects. 2. A fulminating variety occurs in which vomiting is not seen, the patient being precipitated dramatically into the stage of collapse, drowsiness, twitching, convulsions, and fatal coma.

The extreme hypoglycemia and the marked loss of liver glycogen that characterize this syndrome have been recently reported.[18,19] Table 8 shows the blood-sugar levels and liver-biopsy findings in the cases studied. Sir Harold Scott, in his original investigation of the disease in 1915, was of the opinion that all cases were due to eating the unripe ackee (Blighia sapida) and felt that the condition should be known as "ackee poisoning." [20] The recent isolation from the ackee of two compounds, called hypoglycin A and B, which have a marked hypoglycemic activity in experimental animals, has given further support to this theory of etiology.[21]

Materials and Methods

During the last four years a total of 527 needle biopsies of the liver has been performed on 330 hospital patients (Table 1). All were Negroes, apart from a few who were of Chinese or East Indian extraction. Eighty-four patients suffering from veno-occlusive disease were studied, and a total of 173 biopsies was done in this group. Sixty-four were classified as acute when first seen; 6, subacute, and 14, chronic. The initial classification in all cases was based on the clinical picture and the liver-biopsy findings. The ages on admission ranged from 6 months to 50 years, but by far the greater proportion were children under 10 years of age. Although initially used mainly for diagnosis, liver biopsy was later used chiefly to determine the clinical natural history of the disease and in assessing prognosis. A number of patients were readmitted while symptom-free for follow-up biopsies if progression to hepatic fibrosis was suspected. A full postmortem examination was made in 21 out of 26 fatal cases, and it has been adequately confirmed by comparison with postmortem material that the liver-biopsy findings

in these patients are representative of generalized changes throughout the liver.

In the kwashiorkor, marasmic kwashiorkor, and marasmus groups a total of 158 biopsies were performed on 78 patients. Sixteen patients with vomiting sickness (29 biopsies) and 29 patients with diabetes (36 biopsies) were also studied. Three of the latter group showed an unexplained associated hepatomegaly, four showed unusually wide fluctuations in blood-sugar levels and insulin requirements while under treatment, and four were examples of the J-type of insulin-resistant diabetes recently reported from Jamaica by Hugh-Jones.[22] Liver biopsies[21] were also performed on 18 patients with sickle-cell anemia. The spleen was clinically enlarged in all cases, and hepatomegaly was present in all but two. These figures cannot be taken to represent the frequency of clinical enlargement of the liver in sickle-cell anemia in Jamaica, patients with hepatomegaly being more frequently selected for biopsy than those without. All the patients had typical sickle-cell anemia, with persistent anemia, unremitting jaundice, bone changes, and recurrent crisis of varying degree.

Cruickshank has recently described a neuropathic syndrome occurring particularly in Jamaican peasants and presenting a wide range of neurological abnormalities.[23] They include one or more of the following syndromes: upper motor neuron damage, damage to the first sensory neuron, retrobulbar neuropathy, and eighth-nerve deafness. Because of the close similarity to the findings in prisoner-of-war camps and the frequent occurrence among the poorer classes, he concluded that the basis of the disorder was probably nutritional. A total of 19 biopsies was done in 19 patients presenting these neurological findings. In 10 nephrotic patients in whom the serum albumin depletion seemed to be out of proportion to the urinary loss failure of hepatic synthesis of albumin was postulated, and a possible histological explanation was sought by liver biopsy. A single biopsy was also performed on each patient in the following categories: unexplained hepatomegaly or hepatosplenomegaly (47), unexplained splenomegaly (10), unexplained jaundice (16), unexplained anemia (3), and prolonged unexplained pyrexia (5).

Liver-function tests were done on most patients within 24 hours of the biopsy. These included one-minute and total bilirubin, thymol turbidity, serum cholinesterase, serum proteins, alkaline phosphatase, prothrombin estimation, and bleeding and clotting times, as previously reported.[9] The technique of liver biopsy and the precautions taken to minimize the risk of the procedure have also been reported.[9]

Histological Examination.—Part of each biopsy specimen obtained was examined histologically. The specimens were fixed in Helly's fluid, and paraffin sections were cut. Serial sections of each block were studied. Routine examinations included hematoxylin-azophloxine, Mallory's trichrome, reticulin, periodic acid-Schiff, and iron stains. Elastic-tissue, fat, and various other stains were occasionally used.

Chemical Measurements.—In addition to the measurements detailed in this paper, studies of the activity of a number of enzymes have also been made at various times in the course of this work. Some of these results have already been reported. Chemical analyses have been done on one or more biopsy specimens from 109 patients. These fall into four groups: kwashiorkor (including marasmic kwashiorkor), 45 cases; marasmus, 13 cases; veno-occlusive disease of the liver (V. O. D.), 34 cases, and miscellaneous, 17 cases.

Water and fat were measured by weight difference after drying and solvent extraction. The fresh specimen was weighed on a quartz-fiber torsion balance[24] and dried overnight in the cold over phosphorus pentoxide. It was found by experiment that constant weight was reached in this time. The dry specimen was then extracted for at least four hours with hot ether and weighed a third time. In pilot experiments on rat livers it was found that this simple method of extraction leaves behind lipids—presumably phospholipids—amounting to about 2% of the fresh weight of the specimen. This error was not considered to be significant for the purpose of these clinical studies.

Two methods were used for estimating nucleic acids and protein in the dry fat-free tissue: (1) a modification of the method of Schneider,[25] which has already been described in detail[26]; (2) a modification of the method of Schmidt and Thannhauser,[27] similar to that used by Logan, Mannell, and Rossiter.[28] In this method both deoxyribose nucleic acid (DNA) and ribonucleic acid (RNA) are measured by ultraviolet absorption.

For protein and DNA the two methods seem to be comparable, and the results have therefore been treated as one group. For RNA Method 1 gives significantly lower figures than Method 2. There is at present no perfect method for the routine analysis of nucleic acids, particularly on the micro-scale. Fortunately, in liver the discrepancies seem to be much less than in nervous tissue.[29]

Measurements were also made of glycogen content in selected cases (Tables 8, 9, and 10). A part of the biopsy specimen was put at the bedside into tared capillary tubes containing 40% KOH. Glycogen was estimated by titration with ceric sulfate after hydrolysis.[29] Nitrogen was

determined in the KOH digest by Kjeldahl digestion and distillation with micro-Conway units.[80]

Sampling Error.—A biopsy specimen of a few milligrams is a very small sample of the whole liver. Billing et al. have shown that in fatty livers there is a good agreement in fat content between specimens taken from different parts of the same liver.[81] In cirrhosis, however, as would be expected, there is much more variability from one sample to another. For this reason we have in general avoided chemical measurements in cases with extensive fibrosis. The biggest group in our material is made up of fatty livers, in which microscopically the lesion is very uniform. We did not think it justifiable to take more than one sample in vivo at any given time. Therefore the only comparisons that can be made are between two fragments of the same biopsy specimen core. In general such comparisons have shown good agreement in fat content and satisfactory agreement in the enzyme activity of duplicate homogenates.[82] There seems, however, to be much more variability in the results for nucleic acids.

Complications of Liver Biopsy

Most of the complications occurred early in the series and were probably attributable to faulty technique. In two children a shock-like stage developed after biopsy and lasted for two to three hours. This was attributed to pleural shock, there being no clinical evidence of bleeding at the time or subsequently. Three patients suffered sufficient abdominal or shoulder-tip pain to necessitate analgesics, but this usually settled in 24 to 48 hours. Significant hemorrhage occurred in two patients. In one case it ceased after a transfusion of 1 pt. (500 cc.) of fresh blood. A 69-year-old patient died of continued hemorrhage in spite of repeated blood transfusions. At postmortem examination he was found to have a gross cirrhosis, with an extensive anastomotic collateral circulation. One of these vessels had been transfixed by the biopsy needle at a point where the right lobe of the liver was adherent to the diaphragm, preventing retraction of the vessel. Kidney tissue was unexpectedly obtained on three occasions in this series, but there were no untoward effects.

Histological Findings

Veno-Occlusive Disease of the Liver. The essential findings in the acute stage are blockage of the small hepatic veins, the medium-sized veins being somewhat affected and the larger veins being essentially free from lesions. The occlusion or partial occlusion is a result of a subendothelial swelling of intimal tissues, at first apparently mainly consisting of edema but becoming organized later.[10] The block is followed by massive centrilobular congestion, and the macroscopic appearance in this stage is that of a nutmeg liver, enabling the diagnosis of acute V. O. D. to be made on the gross inspection of the liver-biopsy specimen.

We have been unable to demonstrate direct and primary damage of the liver parenchyma; it would appear that the hepatic parenchymal cells perish only secondarily to the congestion and as a direct result of it.

The acute stage may subside clinically, and in follow-up biopsies under such circumstances no trace of the original disease was revealed in many instances except for a mild persistent thickening of the walls of some of the hepatic veins. (When patients died in the acute stage, after a period of progressive liver failure, the histological picture differed only in degree from that described above for the acute stage from which patients recovered.)

The subacute stage is characterized histologically by persistent fibrosis in centrilobular (nonportal) areas. The lumen of the vessels is usually diminished to a varying degree, and as a rule it was not difficult to demonstrate completely occluded vessels in the biopsy specimens. Collateral circulation under such circumstances had developed from coalescence of neighboring sinusoids.

The chronic stage is one of nonportal cirrhosis,[10] clinically indistinguishable from other types of cirrhosis but histologically still rather well characterized, owing to the nonportal distribution of the connective-tissue septa. As far as we can see, this end-result is entirely due to repeated veno-occlusive lesions, with subsequent irreparable damage

to nonportal areas, scarring, formation of collateral circulation, and nodular regeneration.

Kwashiorkor and Marasmus. — Fatty change is commonly seen but is different in degree in kwashiorkor and marasmus.[33] In many cases of kwashiorkor it would appear that every liver cell is filled almost to capacity with fat. The predominant peripheral distribution of the fat and the fact that fat in this peripheral position remains longest in follow-up biopsies during the time the patients undergo appropriate treatment has been described by many investigators and has generally been confirmed also in our material. It has, however, not uniformly been the case.

Eosinophilic degeneration of cytoplasm, with nuclear changes indicating necrobiosis (karyoklasis, pyknosis), were frequently seen, although biopsy specimens usually do not show more than two or three such cells in the entire section studied. Occasionally these were surrounded by histiocytic, lymphocytic, eosinophilic, or polymorphonuclear cells. This type of degeneration showed no predilection for any part of the lobules. Fibrous replacement does not seem to take place after it.

The nuclei of the parenchymal cells commonly showed irregularity in size and shape, and polyploidy, a phenomenon frequently seen in elderly people[34,35] but less common in infants and children. Also there was ample evidence of amitotic division, as well as mitoses, undoubtedly signs of regeneration. Glycogen infiltration of the nuclei[36] occurred, mainly in periportal areas. The portal triads showed a tendency to increased cellularity (mostly histiocytic, lymphocytic, fibrocytic cells, eosinophils) as the patient recovered. In some instances this was accompanied by periportal reticulin increase, causing the portal areas to link up. Proliferation of the bile ducts was only seen in three or four cases.

During the stage of fatty change the sinusoids were compressed. During treatment there was a cellular increase in the sinusoids. The Kupffer cells became hyperplastic and proliferated, while lymphocytes appeared. However, large numbers of lymphocytes, simulating lymphatic leukemia, as reported by Davies,[37] were not seen.

It was reported in 1953 that so far no fibrosis had been seen to develop in our patients in follow-up biopsies done at various times after the initial disease.[38] This point is at present receiving intensive study.

Vomiting Sickness.—In patients in the acute stage the most striking feature is the complete absence of glycogen, most dramatically brought out in the glycogen stains, such as, for instance, the periodic acid-Schiff stain. In hematoxylin-and-eosin sections the cytoplasm looks pale and moth-eaten; the nuclei also appear slightly pale. Small vacuoles containing fat may develop in a matter of few hours to a day. They remain invariably small and do not displace the nucleus toward the periphery of the cell as is seen in marasmus and kwashiorkor.

The glycogen is re-formed under appropriate treatment in those patients who recover, and it has been seen by us to appear histologically in adequate quantity again in a matter of 48 hours. During this period fatty change is apparently unaffected, but in follow-up biopsies one week after the acute episode fat vacuoles had uniformly disappeared completely. Histological sequelae to the acute episode have not been noticed.

Diabetes.—In three patients the liver was clinically enlarged, but liver tissue obtained at biopsy was normal in two and showed only mild fatty infiltration in the other. Five others showed mild degrees of fatty infiltration without hepatic enlargement; and in two insulin-resistant cases no histological changes were seen on biopsy examination.

Sickle-Cell Anemia.—In 9 out of 18 patients with sickle-cell anemia sickled cells were seen in the sinusoids, the liver parenchyma being normal in appearance. In two patients, in addition to sickled cells lying free in the sinusoids, extensive phagocytosis of these cells by the Kupffer cells was also seen. These biopsy specimens also showed a gross

fibrosis of the postnecrotic type. A diffuse fibrosis was seen in three instances. The liver-biopsy findings were normal in the remaining cases.

Nutritional Neuropathies and Nephrosis. The findings in the 19 cases examined were normal, except for one which showed a slight fibrosis and another in which a diffuse lymphocytic infiltration was observed. No histological abnormalities were noted in the 10 nephrotic patients examined by liver biopsy.

Unexplained Hepatomegaly and Other Undiagnosed Conditions.—The contribution of liver biopsy to the diagnosis of unexplained hepatomegaly, splenomegaly, jaundice, pyrexia and anemia are shown in Table 3.

Changes in Clinical Diagnosis as a Result of Biopsy.—These are shown in Table 4.

Other Findings.—Gummata of the liver were found in two patients with a tender unexplained hepatomegaly and strongly positive serological tests for syphilis. A granulomatous lesion was found in a 53-year-old male patient with an unexplained pyrexia. Its significance, however, could not be determined. The marked increase in hepatic glycogen in a child with glycogen-storage disease has been previously reported.[39]

TABLE 3.—*Contribution of Liver Biopsy in Patients with Undecided Diagnosis*

Clinical Findings	Patients, No.	Biopsy Findings	No.	%
Unexplained hepatomegaly	47	Veno-occlusive disease	14	29.8
		Normal liver	12	25.5
		Fatty liver	6	12.8
		Portal cirrhosis *	4	8.5
		Advanced cirrhosis *	3	6.4
		Postneurotic scarring	2	4.2
		Gumma	2	4.2
		Primary or secondary carcinoma	2	4.2
		Specimen inadequate	2	4.2
Unexplained splenomegaly	10	Normal liver	3	30.6
		Portal cirrhosis	4	40.0
		Postneurotic scarring	1	10.0
		Veno-occlusive disease	2	20.0
Unexplained jaundice	16	Normal liver	7	43.7
		Veno-occlusive disease	4	25.0
		Portal cirrhosis	3	18.7
		Infectious hepatitis	2	12.5
P.U.O.	5	Normal liver	4	80.0
		Granuloma	1	20.0
Unexplained anemia	3	Normal liver	3	100.

* Subsequent postmortem examination revealed the presence of a primary carcinoma of liver in one patient in each of these categories.

TABLE 4.—*Change in Clinical Diagnosis as Result of Biopsy*

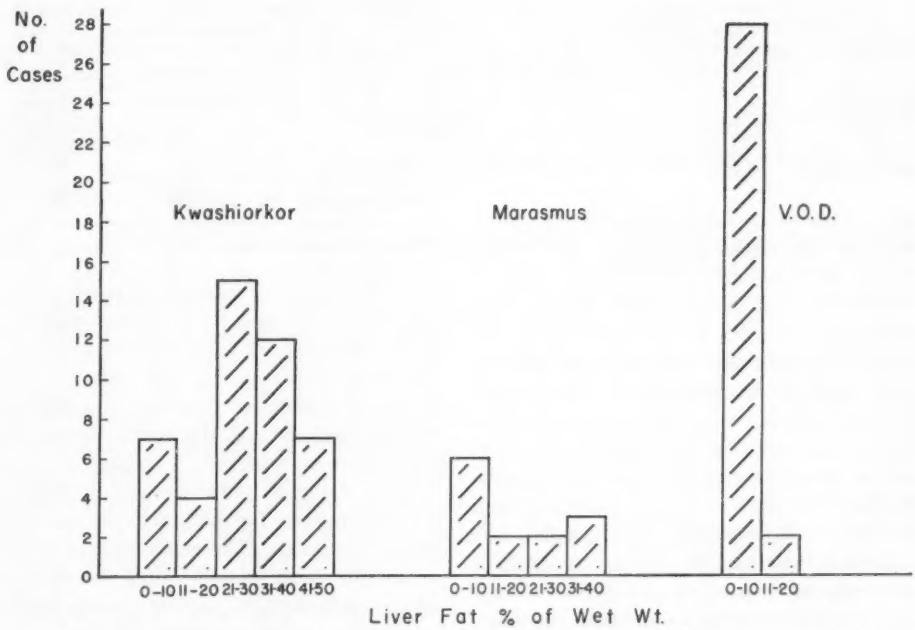
Clinical Diagnosis	Biopsy Diagnosis	Patients, No.
Fatty liver	Normal liver	6
	Veno-occlusive disease	1
	Glycogen-storage disease	1
Primary or secondary neoplasm	Advanced cirrhosis	3
	Portal cirrhosis	1
	Chronic veno-occlusive disease with cirrhosis	2
	Postneurotic scarring	1
Infectious hepatitis	Veno-occlusive disease	1
	Portal cirrhosis	2
Veno-occlusive disease	Normal liver	2
	Advanced cirrhosis	3
	Portal cirrhosis	1
Total		24

A postnecrotic type of scarring was found in three patients with sickle-cell anemia. This lesion was also found in three other patients, 2 men aged 48 and 54 years, with unexplained hepatomegaly, and a woman aged 51 years, with unexplained splenomegaly. It is interesting to note that in neither of these latter cases had there been a preceding history of jaundice or any sufficiently conspicuous illness to indicate the nature of the hepatic disorder.

Chemical Results

Fat Content.—Malnutrition: Only cases are included in which biopsy was done within 14 days of admission to the hospital. Usually three to eight days elapsed before biopsy; during this time the necessary investigations were done, and the child was watched to see if he were in condition for liver puncture. Very many patients take food poorly for at least the first few days in the hospital. Perhaps this is the reason that high levels of liver fat were still found a week or more after admission. One baby had a liver fat content of 38% after 23 days, and another, a fat content of 35% after 15 days. Such cases, however, are exceptional. Serial biopsies have shown that the liver fat may be reduced nearly to normal levels in three or four weeks.[40] This is probably the usual course of events in cases that respond to treatment and begin to take food.

The Figure shows that in kwashiorkor the liver was typically very fatty, whereas in veno-occlusive diseased there was an excess



Distribution of liver fat in kwashiorkor, marasmus, and veno-occlusive disease (V. O. D.).

of fat in only 2 cases out of 30. What is perhaps surprising is that in the group diagnosed as marasmus 6 cases out of 13 had severely fatty livers. In four of these cases the liver was clinically enlarged (liver edge 2 fingerbreadth or more below the costal margin). Conversely, in the kwashiorkor group seven cases had little or no excess liver fat. By hindsight three of these could perhaps be regarded as misdiagnoses.

Perhaps the clearest distinction between kwashiorkor and marasmus in this series is in the incidence of edema, which was present in 40 out of 45 cases of kwashiorkor and in only 1 out of 13 cases of marasmus. This is to be expected, since edema is an important diagnostic criterion of kwashiorkor. This clinical distinction is not reflected in any striking difference between the plasma protein concentrations of the two groups (Table 5).

Jelliffe, Bras, and Stuart,[16] on clinical grounds, separated a subgroup of cases, which they called "marasmic kwashiorkor." These have been summarized separately in Table 5. As far as the degree of edema is concerned, these cases lie midway between kwashiorkor and marasmus. On biochemical grounds, however, it would be difficult to draw any clear dividing line between the groups.

If the cases of kwashiorkor, marasmic kwashiorkor, and marasmus are taken together and classified according to the pres-

TABLE 5.—*Liver Fat and Blood Proteins in Malnutrition and V. O. D.* *

Group	Cases, No.	Edema, Grade	Liver fat, % of Wet Wt.	Serum		Cholinesterase, Units
				Total Protein, Gm./100 Ml.	Albumin, Gm./100 Ml.	
Kwashiorkor	23	2.1	27.5	4.53	2.16	0.29
Marasmic kwashiorkor	19	1.1	26.6	4.65	2.52	0.24
Marasmus	13	0.	16.5	5.25	2.86	0.32
V. O. D. (acute and subacute)	18	--	4.2	6.33	3.05	0.40

* The figures are mean values.

TABLE 6.—*Relation Between Edema and Fatty Liver* *

	With Edema	Without Edema
With fatty liver	37	9
Without fatty liver	6	9

* Edema seems to be more probable in cases with fatty liver ($\chi^2=8.5$, $p<0.01$).

ence of edema on the one hand and of fatty liver on the other, we obtain the results shown in Table 6. There seems to be a definite association between edema and fatty liver ($\chi^2=8.5$, $P<0.01$). Exactly the same result was obtained in an analysis of fatal cases of malnutrition.[41] The nature of this association is not understood; it may be that in cases with fatty liver there is on the average a greater degree of hypoproteinaemia through failure of plasma protein synthesis. Alternatively, as Gopalan has suggested,[42] perhaps in the fatty liver there is a reduced destruction of antidiuretic hormone. Apart from the question of diagnosis, we have tried to detect some relation between the amount of fat and the general condition of the patient or his response. A few cases with very severe fatty infiltration (fat content more than 45%) have died, but numbers are too small for any cause-and-effect relationship to be established. Conversely, many babies with quite severely fatty livers seem to be comparatively well and respond rapidly to treatment. We have therefore come to regard the fat almost as an epiphenomenon. However, as has been pointed out before,[40] removal of fat from the liver is by no means synonymous with recovery from malnutrition.

Other Conditions: Biopsy specimens have been analyzed in a small number of other cases.

Hepatomegaly of Unknown Origin: In five children studied, three were 3 years old or over, that is, above the usual age at which malnutrition is severe. Only one child had a significantly raised liver-fat content (11% of the wet weight), and this case was the youngest of the group (12 months old).

Diabetes: Biopsy specimens were analyzed from five adult cases of diabetes. In three there was a moderate increase of fat (13.5%, 15.7% and 9.1% of wet weight). In the other two the fat content was normal.

TABLE 7.—*Water Content of Liver-Biopsy Specimens*

	Water, Mg./Mg. Fat-Free Solids *	
	Initial Biopsy	Final Biopsy
Kwashiorkor	3.34 (42)	2.94 (18)
Marasmus	3.72 (10)	3.21 (7)
V. O. D. (acute and subacute)	3.35 (14)	3.27 (4)

* No. of cases in parentheses.

Water Content.—In our studies of postmortem material it was found that in malnourished infants the water content of the liver was substantially higher than in children dying of other diseases.[41] This is not confirmed by the measurements made on biopsy specimens. The water content of the initial specimens, taken within a few days of admission to hospital, is only slightly higher than at the end of treatment (Table 7). In acute and subacute veno-occlusive disease there is also no evidence of an increase in water content. It must be admitted, however, that these measurements of water are not very accurate. In spite of all precautions, it seems certain that a significant and variable amount of water must be lost by contact and by evaporation during the manipulations of removing the specimen from the needle, cutting off a piece for histological examination, etc.

Glycogen.—The results in cases of vomiting sickness previously reported are shown in Table 8. Other results obtained are summarized in Table 9. Table 10 shows in a few cases the changes in glycogen and nitrogen content that occurred as a result of treatment. In the cases of kwashiorkor the

TABLE 8.—*Liver Glycogen in Cases of Vomiting Sickness* *

Case	Age, Yr.	Sex	Blood Sugar Mg./100 Ml.	Liver Glycogen, % of Wet Wt.
1	28	M	21	Less than 0.2
2	6	M	19	0.7
3	3	F	15	Less than 0.4
4†	23	F	15	Less than 0.2

† A further liver biopsy specimen taken when the patient had quite recovered showed a liver glycogen value which was within normal limits (7.5%).

TABLE 9.—*Amounts of Liver Nitrogen and Glycogen Found in Various Pathological States* *

Diagnosis	Liver Nitrogen, %	Mg./Mg. Liver Glycogen, Liver Nitrogen	Glycogen (Wet Wt.), %	Cases, No.
Marasmus	2.30±0.68	2.23±1.41	5.14	13
Kwashiorkor	1.69±0.44	2.58±1.08	4.35	10
Veno-occlusive disease	2.40±0.48	1.50±1.11	3.60	22
Diabetes	2.74±0.43	1.30±0.58	3.56	13

* Mean ± standard deviation of mean.

nitrogen concentration was initially low because of the increased fat content. The livers in marasmic cases contain variable amounts of fat, so that some of the initial nitrogen figures in this group are also low.

Statistical analysis shows significantly greater glycogen-nitrogen ratios in marasmus and kwashiorkor than in veno-occlusive disease and diabetes. In all four conditions treatment seems to cause changes in glycogen and nitrogen levels to intermediate values which are presumably closer to normal.

Nucleic Acids and Protein.—In a smaller series of cases (Waterlow and Weisz, 1956), evidence was produced that these malnourished babies have lost a large proportion of their liver protein.[26] This conclusion was based on the finding that the ratio protein nitrogen-deoxynucleic acid phosphorous (N/DNAP) was about 40% lower in initial specimens than after recovery. Losses of protein of the same order were deduced from the results of analysis of the liver in fatal cases.[41] The present series shows the same general trend (Table 4), although the results are not so striking as in the smaller group that was studied earlier. Unfortunately we have no true figure for the "normal" value of the N/DNAP ratio in the human liver. If the values found in the predischarge biopsies are taken as normal, then in the malnourished cases on admission the liver must have lost on the average about 25% of its protein. In veno-occlusive disease, on the other hand, the ratio N/DNAP was not reduced.

TABLE 10.—*The Effect of Treatment on Liver Nitrogen and Glycogen*

Diagnosis	Liver Nitrogen,		Glycogen/Nitrogen		No. Cases,
	Before	After	Before	After	
Marasmus	1.90	2.50	1.65	2.35	5
Kwashiorkor	1.73	2.21	2.62	2.18	5
V. O. D.	2.17	2.15	0.86	1.17	9
Diabetes	2.72	2.94	1.23	1.64	8

Measurements were made on one case of glycogen-storage disease, which has been described fully elsewhere.[39] Unfortunately, liver glycogen was not estimated. It was interesting, however, that although the N/DNA ratio was within the usual limits for malnourished infants (N/DNA=59 mg. per milligram), both nitrogen and DNA per milligram of fat-free dry weight were extremely low (N=1.3 mg. per 100 mg. dry weight, compared with the usual figure of 6 to 8; DNAP=22 μg. per 100 mg., compared with the normal figure of 80 to 100). This suggests dilution by some nonfatty substance, presumably glycogen. The liver contained no excess fat.

It had been hoped that measurement of the N/DNAP ratio in biopsy specimens of the liver might give some measure of the degree of protein depletion of the patient. This hope has not been fulfilled. The reasons for this and the limitations of this approach have been discussed in detail in the previous paper.[26] There is great variability in the results obtained, not only from case to case but even between different pieces of the same biopsy specimen. Experiment has shown that this variation is too great to be explained simply by analytical error, and it is more likely that it is caused by inhomogeneity. The specimens are so small (0.25 to 0.5 mg. of dry weight) that they may easily contain significantly different proportions of portal-tract cells, infiltrating lymphocytes, etc. A further limitation of the method is that the degree of protein deple-

TABLE 11.—*Protein and RNA Content of the Liver, Expressed in Relation to DNA* *

Group	N/DNAP, Mg./Mg.		RNAP/DNAP, Mg./Mg.	
	Initial	Final	Initial	Final
Kwashiorkor	60 (28)	77 (17)	1.91 (31)	2.04 (18)
Marasmus	59 (11)	84 (10)	1.80 (11)	1.80 (10)
V. O. D. (acute and subacute)	74 (13)	75 (6)	1.98 (11)	2.03 (7)

* Measurements on biopsy specimens. Number of cases in brackets.

tion is probably reflected more accurately by changes in muscle than in liver. This has been shown by preliminary observations on autopsy specimens.[43]

In the earlier series a statistically significant reduction was found in the ribonucleic acid (RNA) content of the malnourished liver. In the present study we have not been able to confirm this finding (Table 11). In postmortem material it seems that the reduction in RNA is much more marked in muscle than in liver.[43]

The results of the chemical work on liver biopsy specimens in Jamaica can be summarized very briefly. They confirm the histological finding that in kwashiorkor, and in many cases of marasmus, there is severe fatty infiltration, which often disappears rapidly with treatment, whereas in veno-occlusive disease there is no excess fat. The amount bears no relation to the severity of the malnutrition. Measurement of protein in relation to DNA shows a great reduction of liver protein in malnourished cases, but again this finding is of little value as a guide either to treatment or prognosis.

Comment

In our earlier cases of veno-occlusive disease the abrupt onset of a gross hepatomegaly and tense ascites in a patient presenting no previous history of liver disease and the finding of hepatic venous occlusions on liver biopsy specimens suggested a diagnosis of Chiari's syndrome. It soon became evident, however, that we were dealing with a condition which was unlike most reported cases of this syndrome, i. e., the lesion seemed to be most marked in the smaller branches of the hepatic veins, and thrombosis could not be demonstrated. The mechanical and physiological effects of these lesions, however, must be very similar and account for the close similarity in the clinical pictures. The almost invariable demonstration of similar hepatic lesions in liver-biopsy specimens in subsequent patients admitted with this clinical picture not only confirmed the frequency of this hepatic lesion in Jamaica but also provided a certain method of establishing the diagnosis in mild or doubtful cases. In Table 2 are given the clinical summary, results of liver-function tests, and the liver-biopsy findings in 10 typical cases (six acute, two subacute, and two chronic) of veno-occlusive disease recently reported. In the first three cases the steady improvement in the liver-function tests that paralleled clinical and histological recovery is to be noted.

Apart from its assistance in diagnosis, liver biopsy, more than any other single method, has contributed to our knowledge of the pathogenesis of this disease and its clinical natural history. A recent analysis of 64 cases of acute veno-occlusive disease has shown, largely on the basis of serial liver biopsies, that 19% die early in the disease of hepatic failure due to extensive hepatocellular damage 48% show complete clinical biochemical and histological recovery, and a number continue in the subacute stage for months or years. The remainder pass into the chronic stage either directly or after an interval of apparent clinical recovery.[9]

Serial biopsies have also made it possible to assess the prognosis with a fair degree of accuracy. It has been found that the outcome in acute cases is intimately linked with the degree of centrilobular destruction of the liver cells; in the cases that come to autopsy the lesions are of the same type as in cases that recover, but they are more extensive. The clinical course of the disease is often variable and unpredictable and

sometimes misleading. In a number of cases, for instance, a typical example of which is given in Case 7 (Table 2), there was apparent clinical recovery, with gradual improvement in the liver-function tests, while serial biopsies showed an underlying progressive fibrosis. In still other cases (Case 5, Table 2) is was possible to follow the relentless progression of the disease from the acute lesion to a chronic fibrosis, with congestive splenomegaly and terminal hematemeses in a relatively short period of time. Finally, liver biopsy provides a means of distinguishing the cirrhosis of veno-occlusive disease from other forms of cirrhosis in some cases seen for the first time in the chronic stage (Cases 9 and 10, Table 2).

Liver Biopsy in Diagnosis of Hepatic Fibrosis.—No attempt is made to review the now extensive literature on cirrhosis of the liver in tropical countries. A high incidence has been reported in India, Ceylon, the Dutch East Indies, Malaya, Africa, and the Middle East.[44] Our aim has been restricted to an assessment of the information obtained by liver biopsy in this series in Jamaica. Our findings have been largely in keeping with the observations of Cullinan[45] and Vint,[46] who have already pointed out the frequently mixed nature of the cirrhosis that commonly occurs in tropical areas, and in a number of our cases it has been impossible to assign the cirrhosis found to any definite histological category. Three distinct histological types, however, have been recognized: (1) the nonportal cirrhosis of veno-occlusive disease, (2) portal (Laennec's) cirrhosis, and (3) postnecrotic scarring. The two latter conditions occurred either alone or in association with other conditions.

In the 18 patients with sickle-cell anemia who have been studied, a diffuse fibrosis was found in 6 patients, and a gross scarring, postnecrotic in type, was found in 3. Green and co-workers have reported similar findings from postmortem examination of 21 patients with this disease.[47] They have pointed out that obstruction of hepatic sinusoids by sickle cells and engorged Kuppfer cells in the presence of an often severe anemia are peculiar to sickle-cell anemia and probably account for the hepatomegaly and fibrosis. The high incidence of hepatic fibrosis in this group is in keeping with their suggestion and would indicate that sickle-cell anemia may be an important cause of cirrhosis in areas where sickle-cell disease is common.

A number of cases were seen with syndromes of intermediate types in which it was impossible to make an exact clinical diagnosis. The differentiation of these conditions is important both for diagnostic and therapeutic reasons. In this respect liver biopsy has been particularly useful. Of particular interest has been the demonstration of postnecrotic scarring without clinical evidence of previous liver damage. These cases, though few, are of interest in that they are in man the counterpart of the experimental disease. They also support the suggestion that massive hepatitis may occur in subacute form without the patient being jaundiced. It is likely, as Himsworth points out,[48] that with increasing recognition this more slowly progressive form of postnecrotic scarring will prove to be even commoner. The one positive correlation in all our cases of cirrhosis has been with long-standing malnutrition, and, although its exact role remains unexplained, the presumption is inescapable that it may be an important factor in aggravating, if not in initiating, the disease.

Further Comments.—The number of cases (24) in which the clinical diagnosis was changed as the result of liver biopsy (Table 4) reveals the uncertainty of the clinical diagnosis of liver disease, even when based on a complete history, physical examination, and general laboratory and liver-function tests. The frequent finding of a normal histology in patients with unexplained hepatomegaly, splenomegaly, or jaundice and the high incidence of veno-occlusive disease as a cause of unexplained hepatomegaly (Table 3) are of interest. Biopsy was most apt to be misleading in the presence of hepatic neoplasms by failing to reveal the tumor (Table 3). We have not

thought this a particular disadvantage in so far as the diagnosis soon becomes apparent, and we have tended to avoid this procedure in cases where this diagnosis was strongly suspected.

The finding of relatively large amounts of glycogen in the livers of patients with kwashiorkor and marasmus (Table 9) was not unexpected, particularly in view of their relatively high-carbohydrate diet. It would therefore appear that severe fatty infiltration does not impair the capacity of the liver to store glycogen. No firm conclusions, however, can be drawn from these isolated glycogen estimates in this group or in diabetes, and measurements of turnover rates under various dietary and therapeutic conditions would be necessary.

Zimmerman and co-workers have recently demonstrated the difficulty of correlating the hepatic status either histologically or biochemically with diabetic control.[49] We have been able to confirm this. In three patients the liver was clinically enlarged, but liver tissue obtained at biopsy was normal in two and showed only mild fatty infiltration in another. In five cases there were mild degrees of fatty infiltration without hepatic enlargement, and in three insulin-resistant cases no histological changes were seen on examination of the biopsy specimen. It was also impossible to correlate alterations in liver-function tests which were minimal when present with either hepatomegaly or diabetic control.

It is hardly necessary to attempt to define the relative importance of histological or chemical methods in these studies. In some, e. g., sickle-cell anemia and veno-occlusive disease, the histological findings have been of greater importance. In others, particularly in kwashiorkor, marasmus, and vomiting sickness, the contribution of chemical studies has been more significant. In most cases, however, the findings have been complementary and have helped toward a fuller understanding of the hepatic changes. Chemical measurements, for instance, have shown the unimportance of the degree of fatty infiltration in dictating the clinical picture or the prognosis of kwashiorkor. Parallel histological studies have confirmed these observations by demonstrating its disappearance from the liver, with no residual damage in recovered cases. It is also of interest that we have been unable to demonstrate by serial biopsies the development of hepatic cirrhosis in kwashiorkor and marasmic patients with severe fatty infiltration of the liver, and it would suggest that fatty infiltration per se is an unimportant cause of hepatic cirrhosis in Jamaica, in children, at any rate—a suggestion that has already been indicated by the chemical measurements.

A probably not unimportant contribution of liver biopsy in this series has been the demonstration that the multidisciplined approach is technically feasible. In a number of cases six or seven enzyme assays, reported elsewhere,[32,50] a number of chemical estimations, and histological examinations have been made on the tissue obtained at biopsy. Further work is evidently required, and the results presented here are, in many instances, preliminary studies. We feel, however, that it is along these lines that future clarification of the wide range of hepatic disorders commonly encountered in the tropics particularly but also in temperate areas will be found.

Summary

Five hundred twenty-seven biopsies have been performed on 330 patients. The conditions studied included kwashiorkor, marasmus, veno-occlusive disease of the liver, vomiting sickness, diabetes mellitus, various neuropathies, nephrosis, sickle-cell anemia, and unexplained hepatomegaly, splenomegaly, jaundice, pyrexia, and anemia.

A clinical outline has been given of veno-occlusive disease, kwashiorkor, marasmus, and the vomiting sickness of Jamaica.

The tissue obtained at biopsy was examined histologically in all cases. In a number of cases chemical examination of the liver tissue was also performed. The chemical studies included nucleic acids and protein,

water and fat content, and glycogen estimations.

In the malnutrition group it is shown (*a*) that the degree of fatty infiltration is no indication of the severity or probable outcome of the disease, (*b*) that hepatic protein depletion is severe in these infants but that its degree cannot be quantitatively correlated with the clinical picture or prognosis, (*c*) that fatty infiltration does not apparently interfere with the ability of the liver to store glycogen.

Occlusion of the smaller and medium-sized branches of the hepatic veins is responsible for the hepatomegaly and ascites found clinically in veno-occlusive disease.

The prognosis of veno-occlusive disease is linked with the severity of the associated hepatocellular damage.

Serial biopsies have made it possible to define more accurately the clinical natural history of this disease. In cases that recover the histological appearances of the liver return to normal. When the disease advances to the chronic stage, a progressive nonportal cirrhosis is found to develop in biopsy specimens.

The histological changes in the liver in diabetes are minimal and cannot be correlated with the response to treatment, clinical hepatomegaly, or alteration of liver-function tests.

The hypoglycemia of vomiting sickness is associated with severe depletion of hepatic glycogen, which is rapidly restored by successful glucose therapy.

There is a high incidence of fibrosis of the liver in patients with sickle-cell anemia, and a possible etiological relationship is suggested.

Because of the frequently mixed nature of hepatic cirrhosis in the tropics and the variability of the clinical syndromes presented, liver biopsy is a useful and often essential tool for establishing a correct diagnosis. Its uses in unexplained hepatomegaly, jaundice, and splenomegaly have also been demonstrated. It is also pointed out that liver biopsy often makes it necessary to alter an apparently firmly established clinical diagnosis.

The advantages of simultaneous histopathological and chemical examination of portions of the tissue obtained at biopsy have been stressed.

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Treatment of Experimental Histoplasmosis with Amphotericin B

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Previous work[1-3] has indicated that the drug amphotericin B (an antifungal antibiotic isolated from an unidentified species of Streptomyces) has antifungal activity against Histoplasma capsulatum. This has been shown both in vitro and in experimental infection. Our own previous results as well as the results of other groups have indicated that in spite of the relative insolubility of the drug definite activity against experimental histoplasmosis has been demonstrated.[1-3]

The hamster has been the experimental animal of choice in histoplasmosis in our experience, and for this reason it has been used in the present work to further evaluate the effect of amphotericin B in experimental disease.

Previously the combination of amphotericin B with sulfadiazine had given better results than amphotericin alone. The sulfadiazine alone had little effect in our hands.[1,2]

The relatively insoluble nature of the drug, amphotericin B, was the most serious drawback to its use in our previous experience.[1,2] A new product which was of increased solubility as compared with the first one was used in the present experiment.

Materials and Methods

One hundred fifty hamsters (one hundred thirty-two weighing 30 gm. plus eighteen of slightly greater weight) were given intraperitoneal injections of a 0.5 ml. suspension of yeast cells of our Strain 5 H. capsulatum in saline (100,000 cells per 0.5 ml.). The organism had been grown at 37 C on blood agar for four days. These animals were divided into the following groups: 50 untreated controls; 25 animals treated with amphotericin B (Squibb Lot ST. 191/14/15-6), 1 mg. per day, treatment starting the day after infection; 25 animals treated with amphotericin B, 1 mg. per day, treatment starting on the eighth day after infection; 25 animals treated with amphotericin B, 1 mg., and sulfadiazine sodium (Lederle Lot 3921-237B), 30 mg., per day, treatment starting the day after infection, and 25 animals treated with amphotericin B, 1 mg., and sulfadiazine sodium, 30 mg., per day, treatment starting on the eighth day after infection. The drugs were prepared fresh each day and injected subcutaneously. The last day of treatment was the 18th day after infection, which meant that the two groups starting treatment the day after infection received 18 days and the other groups, 11 days of treatment. As animals died they were discarded, but a representative few were autopsied. Sixty-four days after infection the experiment was terminated by killing all animals still alive, taking cultures from their livers and spleens, and autopsying them, sending tissues for microscopic examination.

Results

The first control animal died on the 12th day after infection. All deaths in the other groups occurring before this were discarded as being due to causes other than histoplasmosis. It is apparent from the Table and the Figure that the treatment had a marked effect on the survival of the hamsters. There was a slight difference in the effect of amphotericin B alone and of amphotericin B and sulfadiazine sodium combined.

The results of the cultures show that the drug apparently sterilized only the animals started on treatment the day after infection.

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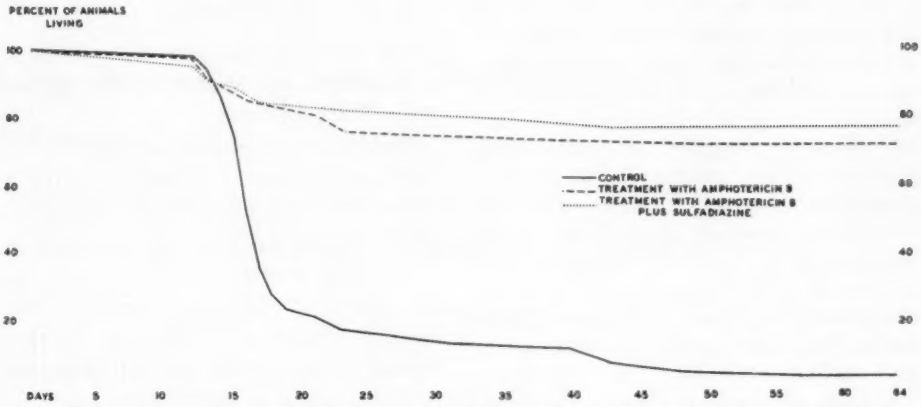

Results of treatment with amphotericin B alone and in combination with sulfadiazine of experimental histoplasmosis in hamsters (Cricetus auratus).

(Those animals had seven more injections than the group starting treatment late.)

No animal died from the treatment per se as far as could be determined, and at autopsy no deposits of drug were noted in the soft tissues of the animals, as was the experience in previous work.[1,2]

Microscopic study of tissues of animals surviving to the end of the experiment revealed that organisms were demonstrable, and organ lesions were observed in all cases which had positive cultures. Those animals whose organs were negative on culture (all surviving early-treatment animals) showed no organ pathology and no cells of H. capsulatum in the tissues.

Comment

It appears from these experiments that amphotericin B either alone or in combination with sulfadiazine sodium is an effective therapeutic agent in experimental histoplasmosis in hamsters. The data indicate that the addition of sulfadiazine sodium improves the effect of the treatment on the infection.

Results of Treatment with Amphotericin B Alone and in Combination with Sulfadiazine Sodium

	Animals Surviving After 64 Days/ Total Infected	%	Positive Cultures from Surviving Animals After 64 Days, %	
			100	
			Late Treatment	Early Treatment
Control	2/50	4		
Amphotericin B *	34/47 †	73	100	0
Amphotericin B+ sulfadiazine sodium	34/44 †	77	95	0

* These groups represent the total of both the early- and the late-treatment groups.
† The denominators represent the total number of animals infected minus those animals dying before the first control died.

The apparent lack of toxicity of this drug for hamsters is of importance and adds to the significance of the findings. The presence of positive cultures from the surviving late-treatment animals is a point of significance. Certainly the most desirable situation would be effective sterilization of all of the animals. However, the present experiment corroborates the work of Louria, Feder, and Emmon's group[3] that under certain conditions sterilization can be achieved. Increased dosage may be the answer. In any case the activity of amphotericin B, enhanced by sulfadiazine sodium, against H. capsulatum has been firmly established.

It is of added interest that the addition of sulfadiazine sodium to the preparation made it less turbid. This may represent an increased amount of amphotericin B in actual solution or finer dispersal of the amphotericin B (if not actually solubilized). Therefore the sodium sulfadiazine may not add any fungistatic or fungicidal effect of its own but act purely by making more of the amphotericin B available to act against the H. capsulatum.

The implications of this experience are that this drug, amphotericin B, is effective in histoplasmosis and because of its apparent low toxicity should be expected to be effective in human histoplasmosis. Since this disease is benign in the overwhelming majority of cases and the clinical course is protean in asymptomatic cases, care will have to be exercised in ascribing clinical effectiveness to this drug. However there are the infrequent cases of progressive disseminated disease which are commonly fatal, and in these this drug may prove to be the first effective treatment.

A white middle-aged man with a right-upper-lobe lesion clinically diagnosed as carcinoma was subjected to thoracotomy in September, 1956, and biopsy of the mass revealed granulation tissue containing organisms morphologically identical with H. capsulatum. Following surgery he responded fairly well but soon developed weakness, malaise, weight loss, and collapse of the right upper lobe. He was given treatment with amphotericin B intravenously, and, despite chills and fever accompanying almost all of the injections, he showed no laboratory or other clinical evidence of toxicity. His downhill course was halted, and, after treatment had been stopped for six weeks, he experienced weight gain, increase in strength, and reexpansion of the right upper lobe. Though it is very difficult to ascribe the clinical improvement to the use of the drug, arrest of a condition usually progressive is very suggestive of effectiveness of treatment.

At present (Nov. 1957) the patient has again bronchial stenosis and on biopsy from the bronchus carcinoma was diagnosed. His course is downhill.

Summary

The results of treatment of experimental histoplasmosis in hamsters with amphotericin B are reported.

The apparent increased solubility of this particular preparation of amphotericin B is emphasized, and the lack of toxicity is pointed out.

This drug should be tried in clinical human histoplasmosis, but care must be exercised in evaluating the effect of the drug because of the protean nature of histoplasmosis.

The Jewish Hospital Association (29) (Dr. Schwarz).

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Drug-Dependent Coombs (Antiglobulin) Test and Anemia

Observations on Quinine and Acetophenetidin (Phenacetin)

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The Coombs (antiglobulin) test as applied to erythrocytes has been especially of value in the identification and study of isoimmune states [1,2] and in acquired hemolytic anemia [3,4] in which an autoimmune state has been proposed.[5,6] Recently Harris [7] described an additional application of the Coombs test to erythrocytes which was dependent on humoral factors in the patient's serum plus the trivalent antimony compound stibophen (Fuadin). This phenomenon, associated with a hemolytic state due to multiple contacts by the patient with the drug stibophen, may be compared with the drug-dependent agglutination of platelets described by Ackroyd [8,9] in the thrombocytopenia induced by allylisopropylacetylcarbamide (Sedormid) and varified for quinidine [10-12] and quinine.[13] It is also of interest that the agglutination of leukocytes dependent on serum factors and a drug, similar to Ackroyd's phenomenon for platelets, has been described.[14]

In the present report we wish to record the encounter of a positive Coombs test for erythrocytes which appears dependent on serum factors plus the drugs quinine and acetophenetidin (phenacetin).

The study began with observations on a patient who developed a fulminant hemolytic anemia upon the second contact with quinine. It was subsequently extended to the serum from 112 normal subjects. More recently studies of a second patient with an anemia associated with the intake of acetophenetidin yielded similar information.

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Method

The Coombs (antiglobulin) test [1,2] was performed as follows: 0.1 ml. of serum, 0.1 ml. of a 2% suspension of erythrocytes, and 0.1 ml. of a solution of the drug to be tested were incubated for one hour in a water bath at 37 C. After this the erythrocytes were washed three times with abundant saline (buffered to pH 6.8-7.0). Following the third wash the test tube was turned upside down and was allowed to drain. The erythrocytes were allowed to settle into the residual saline, and to this was added 0.1 ml. of the antiglobulin serum. The mixture stood for 15 minutes at room temperature and then was spun at 1000 rpm for one minute. The button of erythrocytes was shaken gently, and the test was read grossly; following this the contents were poured onto a glass slide and observed microscopically for stability or lack of stability of the clumps. A solid clump without free erythrocytes was read as 4+; a few major stable clumps with scattered intervening erythrocytes were read as 3+; smaller stable clumps were read as 2+; scattered fine clumps which were stable for five minutes microscopically were read as 1+; other findings were considered negative (Figs. 1 and 2).

The Coombs (antiglobulin) serum used was prepared locally. The serum was prepared in the rabbit, with use of pooled whole human serum as described previously for the preparation of canine antiglobulin serum (whole-serum rabbit serum).[15] This serum gave good results when tested with Rh+ (D+) erythrocytes lightly and heavily coated with incomplete anti-D antibody.

The trypsin-treated erythrocytes were prepared by a modification [16] of the procedure of Morton and Pickles.[17] A 1% solution of trypsin (Difco 1:250) in phosphate buffer (pH 7.4) was stored at —20 C in small aliquots. A 1:10 dilution of this 1% solution in buffered saline was used as a working solution. One part of this trypsin solution

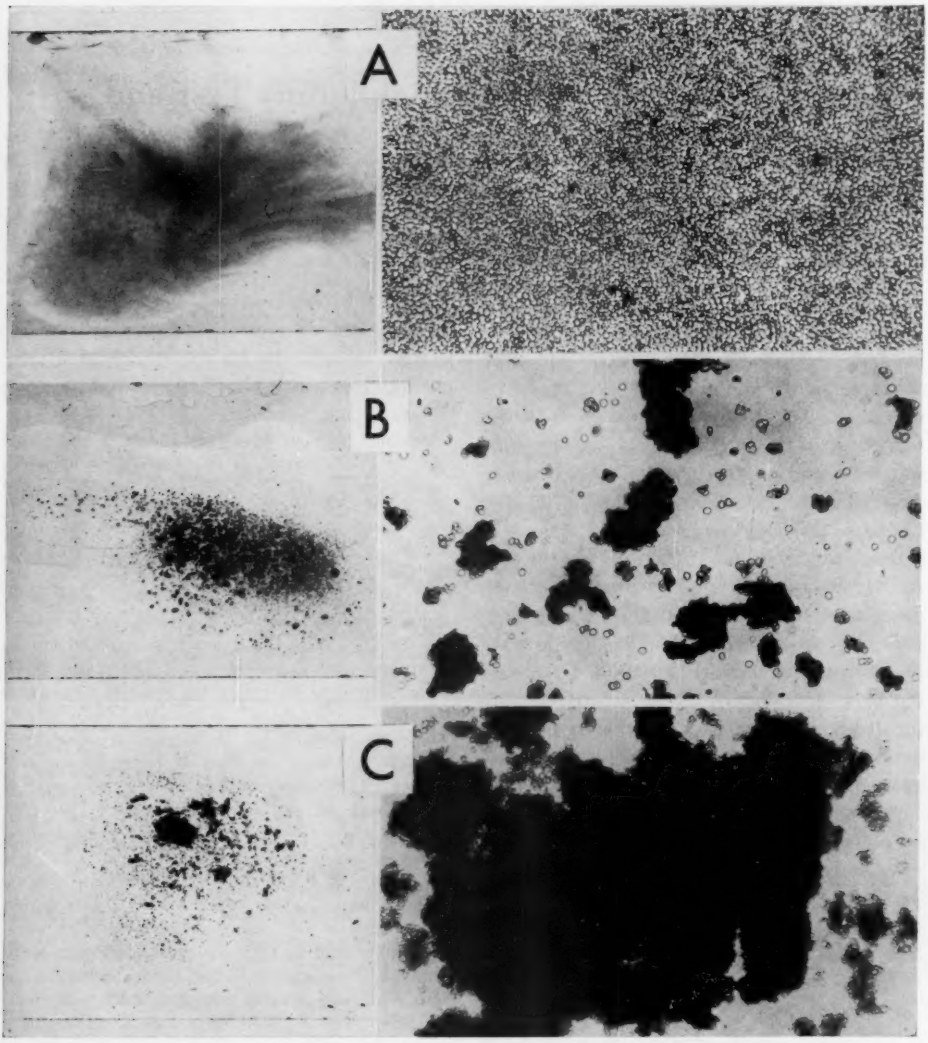

Fig. 1.—Results of the Coombs (antiglobulin) test with use of the serum from a hematologically normal subject (MG) plus compatible erythrocytes and quinine sulfate (50 mg. per 100 cc.). On the left are the gross findings derived by pouring the contents of the test tube onto a glass slide after the test was appraised grossly in the test tube and obtaining a photograph by transillumination; reduced 17% from mag. × 2. On the right are the corresponding microscopic appearances at higher magnification; reduced 17% from mag. × 125. The contents in each procedure and the results were as follows: *A*, inactivated serum plus quinine plus plain RBC; one hour incubation at 37 C; Coombs test; results negative. *B*, inactivated serum plus quinine plus plain RBC plus fresh AB serum; incubation; Coombs test; results 2+. *C*, inactivated serum plus quinine plus trypsin-treated RBC plus fresh AB serum; incubation; Coombs test; results 3+.

was added to 10 parts of a 2% suspension of erythrocytes in buffered saline. The mixture was incubated for 10 minutes at 37 C and centrifuged at 2500 rpm for 10 minutes, and the supernatant was decanted. The erythrocytes were then washed three times in buffered saline and resuspended to a 2% suspension before use.

For inactivation the serum tested was heated to 56 C in the water bath for 30 minutes. When fresh serum was added in the performance of

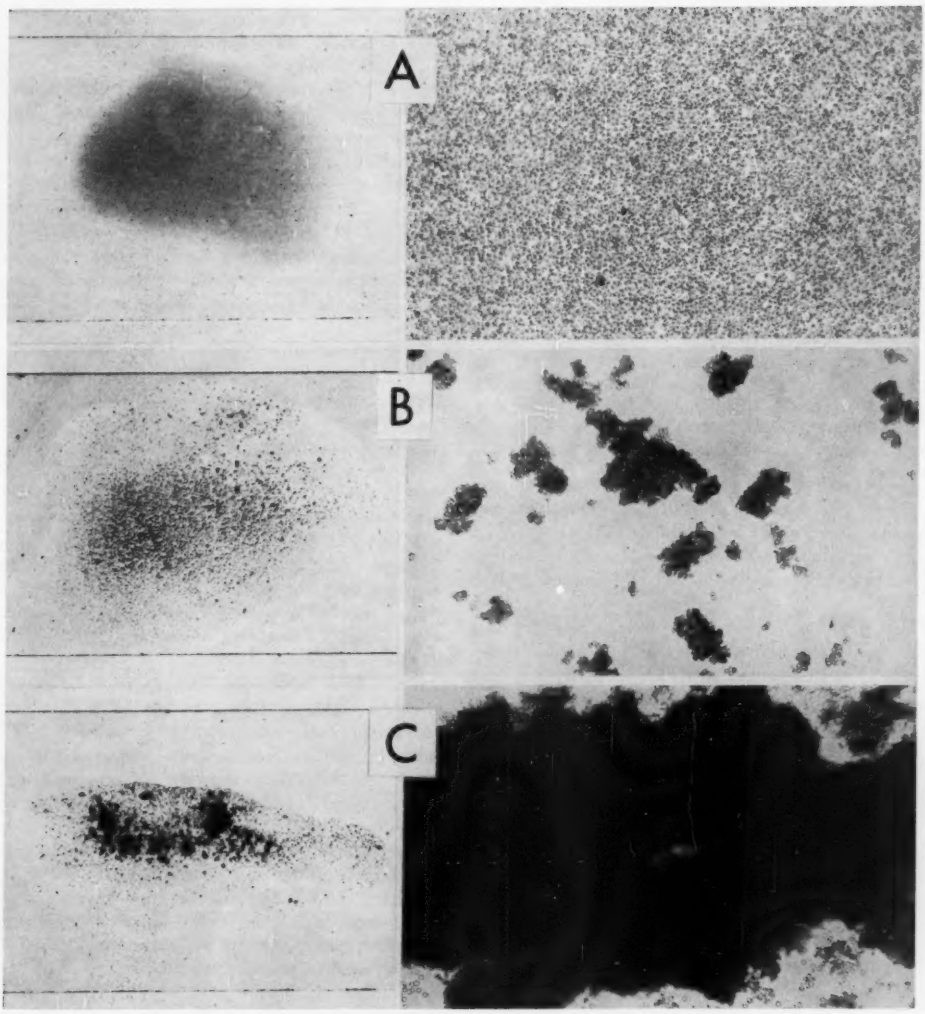

Fig. 2.—Results with use of the serum from Case 2 in the protocol and acetophenetidin (10 mg. per 100 cc.). The scheme is the same as in Figure 1; left side reduced 12% from mag. × 2; right side reduced 12% from mag. × 125. *A*, inactivated serum plus acetophenetidin plus plain RBC; incubation; Coombs test; results negative. *B*, inactivated serum plus acetophenetidin plus plain RBC plus fresh AB serum; incubation; Coombs test; results 1+. *C*, inactivated serum plus acetophenetidin plus trypsin-treated RBC plus fresh AB serum; incubation; Coombs test; results 3+.

the Coombs test the serum from a Type AB donor which was shown to be compatible (directly and by the Coombs test) with the erythrocytes tested was collected and used within four hours.

The quinine sulfate and acetophenetidin used in the tests were obtained from the hospital pharmacy. The quinine sulfate was used in concentrations varying between 50 and 12.5 mg. per 100 ml. of buffered saline, while the acetophenetidin was tested in concentrations varying between 10 and 1.25 mg. per 100 ml. of buffered saline.

There were three sources for the serums tested. The first serum was obtained from a patient who developed a fulminant hemolytic state upon the second known contact with quinine. The second serum was derived from a patient who developed a severe and acute anemia following contact with acetophenetidin. These cases are described below.

The third source of serum were healthy blood donors and laboratory personnel. The serums from the latter source were tested with quinine sulfate.

Fresh serum from 109 blood donors and from 3 of the laboratory personnel were tested as follows: 0.1 ml. serum, 0.1 ml. 2% suspension of trypsin-treated Type O Rh+ erythrocytes, and 0.1 ml. of a quinine sulfate solution containing 50 mg. per 100 ml. were incubated at 37 C for one hour; after this the Coombs test was conducted as described above.

All serums yielding positive results were subjected to multiple control procedures. The control procedures are given in the Tables. In all instances wherein a positive Coombs test developed the test was negative under the same circumstances except for the absence of the drug. Thus, all positive tests reported were demonstrated to be dependent on the drug used.

The platelet and reticulocyte counts were performed according to the alcohol cresyl blue wet preparation.[18] Sickling with sodium metabisulfite was performed as described by Ham.[19] The serum hemoglobin concentration was determined by the modification of Wu and Bing's method as described by Crosby and Furth.[20] The serum bilirubin was determined and fractionated according to Malloy and Evelyn.[21] The spontaneous hemolysis was obtained by Dacie's method.[22] The mechanical fragility was determined according to Shen, Castle, and Fleming.[23] The immediate and incubated osmotic fragility were performed as described by Suess and co-workers.[24] The life span of the erythrocytes in Case 1 was estimated with the radiochromium method.[25]

The Ackroyd test[8,9] for platelet agglutinins in the presence of a drug or chemical was performed by using the procedure of Harrington[26] for platelet agglutinins in the presence of the drug. Leukoagglutinins were checked for in accordance with Dausset's method.[27]

Report of Cases

This report is primarily based on two cases.

CASE 1 (Hemolysis and Quinine Intake).—A 26-year-old Negro woman was admitted with a two-day history of general malaise, frontal headache, and cramping lower abdominal pain. Shortly after the onset of the pain vaginal bleeding began and soon progressed to a profuse flow. The last previous menstrual period had been two months before. A diagnosis of threatened abortion was made.

The day before admission nausea and vomiting were accompanied by fever and chilliness. Yellow sclerae were noted by the patient on the morning of admission. Prominent malaise and muscular aches prompted the patient to go to the hospital.

The past history seemed significant. One month previously the patient ingested "3 oz." of quinine in an attempt to induce abortion. One week prior to the onset of symptoms the patient took a proprietary quinine-containing preparation because of symptoms compatible with an upper respiratory infection. The total quantity of this material taken could not be ascertained. No other drug intake was admitted except for one acetylsalicylic acid tablet (aspirin) and a sedative given the day before admission by a private physician. There was no indication of intrauterine manipulation.

Upon examination the patient appeared acutely ill and restless. The B. P. was 105/70 mg. of Hg; pulse rate, 84 per minute; respiration, 16 per minute, and oral temperature, 98 F. The sclerae were markedly icteric. A small subconjunctival hemorrhage was present on the right. The fundi were normal. The lungs were clear. The heart did not appear enlarged, but a Grade 2 systolic murmur was heard at the base. The liver was tender and palpable 3 cm. below the costal margin. The spleen was not palpable. Pelvic examination showed a dilated cervical os oozing blood. The extremities were normal. There was no purpura. The neurologic examination was normal.

The initial laboratory findings included hemoglobin, 5.3 gm. per 100 cc. of blood; WBC count, 76,000 per cubic millimeter, with a marked left shift of the differential count; urine of black color, free of cellular elements but containing 4+ protein; serum sodium, 130 mEq. per liter; serum potassium, 4.1 mEq. per liter; blood urea nitrogen, 94 mg. per 100 cc.; plasma CO_2-combining power, 19 mEq. per liter, and plasma chloride, 87 mEq. per liter. Later additional tests indicated negative blood culture; Kline test, negative; cephalin-flocculation test, 3+ in 24 hours and 3+ in 48 hours; alkaline phosphatase of serum, 4.4 Bodansky units; plasma prothrombin, 11 seconds (Quick's one-stage method).

The osmotic fragility curves were abnormal. While the control curve was typically sigmoid (beginning at 0.5 gm. of NaCl per 100 cc. and reaching 90% hemolysis at 0.4 gm. of NaCl per 100 cc.), the patient's erythrocytes yielded a biphasic curve. The immediate curve began at 0.6 gm. of NaCl per 100 cc., reached 50% hemolysis at 0.5 gm. of NaCl per 100 cc. and 90% hemolysis at 0.25 gm. of NaCl per 100 cc. The incubated osmotic fragility yielded a prominent "tail," beginning at 0.8 gm. of NaCl per 100 cc. and reaching 50% hemolysis at 0.5 gm. of NaCl per 100 cc. The latter was considered consistent with the presence of spherocytosis.

The patient's erythrocytes were tagged with radiochromium on the day of admission. After

TABLE 1.—*Hematologic Observations in Case 1 on Day of Admission, Sept. 10, 1956*

Study	Value
RBC	1.8 ml./cu. mm.
Hb	4.6 gm./100 cc.
Hematocrit	12%
MCV	66.5 cu.μ
MCH	25.7μg.
MCHC	28.7%
Reticulocytes	3.4%
Sickling (Na metabisulfite)	Negative
Serum Hb	224 mg./100 cc.
Serum bilirubin	
Direct	19 mg./100 cc.
Total	30 mg./100 cc.
Direct Coombs test	Negative
Blood smear	Atypical poikilocytosis & scattered spherocytes
Heinz bodies	None present
Spontaneous hemolysis (48 hr.)	Hb plasma 364 mg./100 cc.
Mechanical fragility RBC	
Immediate	18%
Incubated 37C 24 hr.	34%
Platelet count	
Direct	36,000/cu.mm.
Indirect	141,000/cu.mm.
WBC count (corrected)	74,800/cu.mm.
Nucleated RBC/100 WBC	5
Differential count WBC	
Segmented neutrophils	78%
Band neutrophils	13%
Monocytes	2%
Lymphocytes	7%
Bone marrow smear (500 cells)	
Myeloblasts	0.4%
Myelocytes	27.0%
Young neutrophils	13.8%
Band neutrophils	12.2%
Segmented neutrophils	15.8%
Eosinophils	1.4%
Lymphocytes	8.0%
Plasma cells	2.2%
Pronormoblasts	0.6%
Basophilic normoblasts	5.2%
Polychromatic normoblasts	5.2%
Orthochromatic normoblasts	8.2%
M:E ratio (bone marrow smear very cellular, megakaryocytes ample but showing poor platelet differentiation)	4.2:1

mixing the radioactivity dropped to 70% within 10 hours, to 55% by the second day, and to 40% by the fifth day. These findings supported further an active hemolytic process.

Platelet agglutination tests (Harrington's procedure) with use of patient's serum and platelet-rich plasma with and without the addition of quinine were negative.

The patient's inactivated serum plus leukocytes with and without the addition of quinine yielded no evidence for leukoagglutinins (Dausset's modified method).

Course.—Antibiotics were begun but were discontinued when the blood culture was negative. Blood transfusions and prednisone (Meticorten), 30 mg. daily, were given. There was no urine. On the fourth day the patient became lethargic while on conservative management for the renal failure. On the seventh day the blood urea nitrogen was 326 mg/100 cc. and the serum potassium was 8 mEq. per liter. Dialysis with a Skeggs-Leonards artificial kidney was instituted. Respiratory distress developed the following day, and the patient died on the eighth day (Sept. 18, 1956).

CASE 2 (Anemia and Acetophenetidin Intake).—A 17-year-old Negro girl developed an upper respiratory infection and sustained trauma to the jaw 10 days before admission. An injection of penicillin caused urticaria. Relief of this followed a dose of tripelennamine (Pyribenzamine). For discomfort she was given a preparation containing phenobarbital and acetophenetidin. During the following three days the urine became dark "like tea" and the sclerae became icteric. Weakness became pronounced, and on the day of admission she fainted.

The B. P. was 120/60 mm. of Hg; pulse rate, 124 per minute; respirations, 16 per minute, and oral temperature, 98.4 F. The physical examination, except for pallor, was negative.

Course.—The patient was in the hospital seven days, during which there was steady improvement. The first day she received 1000 cc. of blood. She was given prednisone, 15 mg. daily. The hemoglobin was 8.7 gm/100 cc. on the second day and 10 gm. on the third day. The reticulocytes were 4.0% on the fourth day and 5.7% on the sixth day. The WBC dropped to 13,800 on the fourth day, with a differential count of 68% segmented neutrophils, 4% band neutrophils, 2% myelocytes, 1% basophils, 15% lymphocytes, and 8% eosinophils. The patient was discharged improved seven days later, at which time the hemoglobin was 12.4 gm/100 cc. and the WBC, 10,000 per cubic millimeter.

TABLE 2.—*Hematologic Data in Case 2*

Study	Value
RBC	1.3 ml./cu mm
Hb	4.0 gm./100 cc
Hematocrit	12%
Reticulocytes	0.4%
MCV	91 cu.μ
MCH	31 μg.
MCHC	34.4%
WBC	36,950/cu. mm.
Differential count	
Segmented neutrophils	55%
Band neutrophils	13%
Young neutrophils	14%
Myelocytes	2%
Eosinophils	1%
Monocytes	3%
Lymphocytes	12%
Bone marrow smear (500 cells)	
Myeloblasts	0.8%
Myelocytes	8.0%
Young neutrophils	12.6%
Band neutrophils	17.0%
Segmented neutrophils	12.2%
Eosinophils	5.8%
Basophils	0.2%
Lymphocytes	5.4%
Plasma cells	2.6%
Pronormoblasts	2.2%
Basophilic normoblasts	5.6%
Orthochromatic normoblasts	12.6%
Polychromatic normoblasts	15.8%
M:E ratio (adequate number of megakaryocytes)	1.8:1
Sickling preparation (Na metabisulfite)	Negative
Blood culture	Sterile
Kline test	Nonreactive
Serum bilirubin	1 mg./100 cc.
L. E. preparation	Negative
Urinalysis	Normal

TABLE 3.—*Tests Utilizing Serum of Case 1* *

Serum Date	Date Serum Used	Pt. Serum, Cc.	Pt. RBC, 2 %	Quinine Sulfate, 50 Mg./100 Cc.	A, Rh+ RBC, 2 %	Fresh AB Serum, Cc.	Trypsin RBC, 2 %	Inactivated AB Serum, Cc.	Direct Results	Coombs Test (Indirect)
9/10/56	9/10/56	0.1	0.1	0.1					Total hemolysis	
9/11/56	9/11/56	0.1	0.1	0.1					Total hemolysis	
9/17/56	9/17/56	0.1	0.1	0.1					Partial hemolysis	Total hemolysis
9/17/56	9/17/56	0.1		0.1	0.1				Total hemolysis	
9/11/56	9/17/56	0.1	0.1	0.1					Negative	Negative
9/17/56	9/28/56	0.1		0.1	0.1	0.1			Negative	1+
9/17/56	8/2/56	0.1			0.1	0.1			Negative	Negative
		0.1			0.1	0.1			Negative	Negative
		0.1		0.1	0.1	0.1			Neagtive	Negative
		0.1		0.1	0.1				Negative	Negative
9/18/56	10/2/56	0.1		0.1		0.1	0.1		Negative	2+
9/18/56	10/2/56	0.1		0.1			0.1		Negative	Negative
9/18/56	10/2/56	0.1			0.1		0.1	0.1	Negative	Negative
9/18/56	10/2/56						0.1			Negative
9/18/56	10/2/56			0.1			0.1		Negative	Negative
9/18/56	10/2/56			0.1		0.1	0.1		Negative	Negative

* The first 3 samples lysed erythrocytes in the presence of quinine. This phenomenon disappeared when the serum was stored in the icebox for 1 wk. Aged serum + fresh AB serum yielded a weakly positive indirect Coombs test in the presence of quinine. Trypsin treatment of the erythrocytes made the results stronger. Various negative control procedures are given.

Results

Figures 1 and 2 depict the gross and microscopic appearance of erythrocytes subjected to the Coombs (antiglobulin) procedure as considered in this discussion. Negative and positive results (1+ to 3+) are shown in the Figures.

CASE 1.—The results of the various tests are summarized in Tables 3 and 4. On the day of admission (Sept. 10, 1956) the direct Coombs test was negative. Although this finding differed from that reported by Harris [7] in the case of stibophen sensitivity, it was decided to proceed with observations comparable to those recorded by Harris.

TABLE 4.—*Aged Patient Serum Plus A,Rh+ RBC Plus Fresh AB Serum Plus Quinine* *

Serum Dilution	Concentration Quinine Sulfate, mg./100 cc.					
	Plain RBC			Trypsin RBC		
	50	25	12.5	50	25	12.5
1	1+	1+	±	2+	1+	1+
2	1+	1+	±	2+	2+	1+
4	1+	1+	±	1+	1+	1+
8	1+	1+	±	1+	1+	1+
16	1+	1+	±	1+	1+	1+
32	1+	±	—	1+	1+	1+
64	1+	±	—	1+	1+	1+
128	±	±	—	1+	1+	1+
256	±	—	--	±	1+	—
512	±	—	—	—	1+	—

* Direct observations negative, then indirect Coombs test: Titration with the Coombs test by serial dilution of the serum with saline with 3 concentrations of quinine. Plain and trypsin-treated erythrocytes were used. Negative controls (Coombs test) included tests on trypsin-treated RBC, trypsin-treated RBC + quinine, patient serum + trypsin-treated RBC + quinine in concentrations of 50, 6.25, and 3.1 mg./100 cc.

Serum collected on the second day (Sept. 11, 1956) was stored in the refrigerator (4 C) for six days and then tested. Originally this same batch of serum had caused total hemolysis in the presence of quinine,

Fresh serum obtained on the day of admission prior to blood transfusions plus a suspension of the patient's erythrocytes plus quinine sulfate upon incubation for one hour at 37 C was associated with complete hemolysis. In the absence of the drug hemolysis did not occur. An additional batch of fresh serum obtained on the second day after the blood transfusions gave similar results. By the seventh day the combination of fresh patient's serum plus patient's erythrocytes plus quinine yielded partial hemolysis on incubation. The remaining erythrocytes withstood a triple-washing with saline but when the Coombs (antiglobulin) serum was added in attempting to perform the Coombs test the remaining erythrocytes underwent complete hemolysis. Fresh patient's serum plus compatible erythrocytes (A_1 Rh+) plus quinine gave rise to complete hemolysis on incubation.

TABLE 5.—*Tests with Use of Serum of Case 2**

Serum Date	Date Serum Used	Pt. Serum, Cc.	Inactivated Pt. Serum, Cc.	Pt. RBC, 2 %	Acetrophenetidin, 10 Mg./100 Cc.	O, Rh+ RBC, 2 %	Fresh AB Serum, Cc.	Trypsin RBC, 2 %	Inactivated AB Serum, Cc.	Direct Results	Coombs Test Indirect
11/13/56	11/13/56	0.1		0.1	0.1					Neg.	1+
11/13/56	11/13/56	0.1						0.1		Neg.	Neg.
11/13/56	11/13/56	0.1			0.1			0.1		Neg.	3+
					0.1			0.1		Neg.	Neg.
								0.1			Neg.
11/13/56	1/14/57	0.1		0.1						Neg.	Neg.
11/13/56	1/14/57	0.1		0.1	0.1					Neg.	Neg.
11/13/56	1/14/57	0.1						0.1		Neg.	Neg.
11/13/56	1/14/57	0.1			0.1			0.1		Neg.	±
11/13/56	1/14/57		0.1	0.1	0.1					Neg.	Neg.
11/13/56	1/14/57		0.1		0.1			0.1		Neg.	1+
11/13/56	1/14/57		0.1	0.1	0.1		0.1			Neg.	3+
11/13/56	1/14/57		0.1		0.1		0.1	0.1		Neg.	3+
11/13/56	1/14/57		0.1	0.1	0.1				0.1	Neg.	Neg.
11/13/56	1/14/57		0.1		0.1			0.1	0.1	Neg.	±
				0.1	0.1		0.1			Neg.	Neg.
				0.1	0.1		0.1			Neg.	Neg.
					0.1		0.1	0.1		Neg.	Neg.

* The Coombs test was weakly positive when fresh serum and drug were incubated with erythrocytes. Trypsin treatment potentiated the phenomenon. Inactivation of the serum removed the phenomenon, which then returned upon the addition of fresh AB serum.

but after storage in the refrigerator no changes occurred when the patient's erythrocytes, aged serum, and quinine were incubated. After this incubation the Coombs test was negative. Fresh serum was not added to this system.

A second batch of serum (Sept. 17, 1956) was aged in the refrigerator and used 11 days later. This also gave negative results in the presence of compatible erythrocytes and quinine, both directly and with the Coombs test. However, when fresh AB serum was added to this system (aged patient's serum plus compatible RBC plus quinine plus fresh AB serum) the direct test was negative upon incubation for one hour but the Coombs test was slightly positive (1+). The negative controls included the same erythrocytes plus fresh AB serum, the erythrocytes plus AB serum and quinine, and the erythrocytes and quinine.

Another batch of patient's serum, collected on the eighth day (Sept. 18, 1956), stored in the refrigerator (4 C), and used 14 days later, gave negative results directly upon incubation and with the Coombs test when mixed with compatible trypsin-treated erythrocytes and quinine. But when fresh AB serum was added to this system the direct test was negative and the Coombs test was positive (2+). The same system using inactivated fresh AB serum gave negative results. Thus, a thermolabile ingredient of fresh serum was required for the positive Coombs test. Negative results were obtained with controls consisting of trypsin-treated erythrocytes incubated for one hour, the same cells incubated with fresh serum and quinine, and the same cells incubated with quinine.

Aged patient's serum plus fresh AB serum plus quinine plus compatible erythrocytes were cross titrated (Table 4). Positive results (1+) were obtained to the seventh tube of serial dilution when 50 mg. per 100 cc. of quinine sulfate was used and to the fifth tube when 25 mg. per 100 cc. was used. Positive results were not obtained with 12.5 mg. of quinine per 100 cc. of solution. These results were potentiated when the same erythrocytes treated with trypsin were used in the same system.

Summary (Case 1).—A fulminant hemolysis occurred upon a known second contact with quinine. Early in the course quinine and fresh patient's serum caused hemolysis in vitro. Aged serum gave negative results, but the addition of fresh AB serum resulted in a positive Coombs (antiglobulin) test. Trypsin treatment of the erythrocytes potentiated the results of the Coombs test. Inactivation of the fresh AB serum nullified the positive results obtained with aged patient's serum.

Thus, a drug-dependent (quinine) hemolysis and a positive Coombs test were

TABLE 6.—*Fresh Patient Serum Plus Trypsin O,Rh+ Plus Acetophenetidin* *

Serum Dilution	Concentration of Phenacetin in Buffered Saline, Mg./100 Cc.				
	20	10	5	2.5	1.25
1	2+	3+	2+	2+	2+
2	2+	2+	2+	2+	2+
4	1+	3+	2+	2+	2+
8	1+	2+	1+	1+	1+
16	—	—	—	—	—
32	—	—	—	—	—

* Direct observations negative, then indirect Coombs test: Titration with the Coombs test by serial dilutions of the serum of Case 1 with 5 concentrations of acetophenetidin. Negative controls (Coombs tests) included tests on trypsin-treated RBC, trypsin-treated RBC + acetophenetidin, serum plus plain RBC + acetophenetidin (±), and serum + trypsin-treated RBC and no drug.

TABLE 7.—*Observations with Serums from Normal Subjects* *

Coombs Test	No.
4+	2
3+	4
1+	12
Negative	93
Agglutination on incubation	1
Total	112

* Fresh serum from healthy donors was incubated with trypsin-treated RBC and quinine, then the Coombs test was performed. The serums giving positive results yielded negative findings in the absence of the drug.

demonstrated with the patient's serum. Moreover, the Coombs test was dependent in part on factors in the serum which were storage-labile and thermolabile.

CASE 2.—The results of various tests are summarized in Tables 5 and 6.

The patient's fresh serum plus a suspension of the patient's fresh erythrocytes plus acetophenetidin (10 mg. per 100 cc.) upon incubation gave negative direct results but a positive Coombs test (1+). When the same system utilized trypsin-treated compatible erythrocytes the Coombs test became more strongly positive (3+). Various control variations with use of trypsin-treated erythrocytes gave negative results. Inactivated patient's serum plus acetophenetidin plus erythrocytes gave a 1+ Coombs test. The addition of fresh AB serum to this system made the Coombs test stronger (3+). When the fresh AB serum was inactivated the Coombs test became negative.

Fresh patient's serum plus acetophenetidin plus compatible trypsin-treated erythrocytes (O Rh+) were cross titrated. Positive results were obtained to the fourth tube of serial dilutions of the serum and with 1.25 to 20 mg. of acetophenetidin per 100 cc. of solution.

Summary (Case 2).—An anemia developed during the intake of acetophenetidin. Certain characteristics of a hemolytic anemia were present, such as jaundice, dark urine, rapid and severe anemia, leukocytosis, and erythroid hyperplasia of the bone marrow. The patient's fresh serum plus compatible erythrocytes plus acetophenetidin gave a positive Coombs test (1+). The test became stronger when trypsin-treated erythrocytes were used. Fresh AB serum potentiated the phenomenon.

Observations with serums from normal subjects (fresh serum plus trypsin-treated RBC plus quinine) were as follows: The results are summarized in Table 7. Of the 112 serums tested, 1 yielded direct agglutination, 2 gave a 4+ Coombs test, 4 yielded a 3+ result, and 12 revealed a 1+ Coombs test. The remaining 93 serums gave negative results. All positive results were controlled by the demonstration of negative Coombs test when the same serum and trypsin-treated erythrocytes were incubated in the absence of the drug.

Comment

There are increasing indications that drugs effect certain disturbances through the means of an immune mechanism. In hematologic complications due to drugs such a mechanism has been supported for thrombocytopenia,[8-13] leukopenia,[14,28] and hemolytic anemia.[7,29] The present study identified an erythrocytic agglutinative phenomenon which was dependent on serum factors and a drug (quinine or acetophenetidin). The intake of these drugs had been associated with a hemolytic anemia. The association suggests strongly the presence of an immune mechanism in the pathogenesis of the hemolysis due to these drugs in these patients.

Early in the course of the quinine-induced hemolysis the addition of quinine to the combination of patient's or donor's erythrocytes and patient's serum caused hemolysis in the test tube. Later the phenomenon became of the agglutinative type.

The agglutinative phenomenon was unique in that it demanded three general factors and was demonstrated only by means of the Coombs (antiglobulin) procedure. The three general factors consisted of erythrocytes (patient's or compatible from a donor), serum factors which were stable on storage and to temperature (56 C), and serum factors which were labile to storage and temperature (56 C). (By storage-labile is meant labile to storage at icebox temperature [4-10C] for one week or more.) The labile factors could be restored with fresh AB serum and in this sense resembled complement or certain of its components. The stable factors assisted in the agglutination of erythrocytes affected by the Coombs serum and in this sense resembled incomplete antibodies.

The positive Coombs test herein described was more prominent when developed with a Coombs serum prepared in this laboratory with pooled whole human serum. The test was weaker when conducted with antiglobulin serum prepared by commercial suppliers. Moreover, the test became stronger when trypsin-treated erythrocytes were used.

The encounter of quinine-dependent positive Coombs test (using whole-serum Coombs serum) in a substantial number of healthy blood donors was unexpected. Such findings do not necessarily minimize the immune pathogenetic mechanism proposed for Case 1. Ackroyd encountered drug-dependent (allylisopropylacetylcarbamide) agglutination of platelets in subjects who were not thrombocytopenic. Moreover, when Harris [7] transfused into a nonanemic recipient the plasma from the patient exhibiting the stibophen-dependent positive Coombs test (passive transfer) and subsequently gave the recipient stibophen, the Coombs test became positive but no anemia ensued. It is conceivable that prior contact with quinine or compounds with related chemical structures may have stimulated the phenomenon in the donors without causing an anemia. Additional observations are needed in this general area.

Summary

Two cases of hemolytic anemia associated with the intake of the drugs quinine and acetophenetidin (phenacetin), respectively, are presented. A positive Coombs (antiglobulin) test was observed when the patient's serum was incubated with the patient's or a compatible donor's erythrocytes in the presence of the corresponding drug. This erythrocytic agglutinative phenomenon was dependent on stable factors in the patient's serum which resembled incomplete antibodies and labile factors which may have represented complement. The labile factors were reinstituted by the addition of fresh AB serum. The Coombs test was potentiated by the use of trypsin-treated erythrocytes. The test was conducted with the use of whole-serum Coombs serum. The positive Coombs test suggests an immune mechanism as operating in the pathogenesis of the anemia. The mechanism has been observed in healthy adults in the absence of anemia, but this is in keeping with similar observations previously presented concerning immune mechanisms related to drugs.

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The Electromyogram in Myxedema

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Slowing of the deep tendon reflexes has been recognized for many years as a characteristic phenomenon of untreated myxedema.[1] On palpation of the tendon during reflex motion the slowness of response is particularly apparent in the relaxation phase, and "delayed relaxation" is a useful diagnostic sign of myxedema, since it does not appear with any regularity in any other disease. The biceps brachii and the Achilles tendon reflexes are most convenient for this demonstration. Lambert and co-workers[2] were able to record by means of special apparatus the mechanical events of the ankle jerk in myxedema and other metabolic abnormalities. Both contraction and relaxation were significantly prolonged, the latter often markedly so, and beyond the range of normal in 77% of patients with myxedema. In contrast, all patients with low basal metabolic rates but without myxedema showed normal reflex patterns. All myxedematous patients, including those whose initial reflex time was at the upper limit of normal, showed shortening of reflex time after treatment.

The pathogenesis of the delayed tendon reflexes in myxedema is unknown. The present electromyographic study was undertaken to determine if in this disease abnormal reflexes are associated with abnormalities of the intrinsic electrical properties of the neuromuscular apparatus.

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Material and Methods

Twenty consecutive patients with frank myxedema, all of whom showed a characteristic delayed relaxation of the biceps brachii tendon reflex, were studied by electromyography before or shortly after the institution of appropriate therapy. Of these, six were restudied after treatment had resulted in the return to euthyroidism and the abnormal reflexes had disappeared. For comparison, six patients were studied who had thyrotoxic myopathy so severe that they required assistance to raise themselves from the supine position. Normal electromyographic findings in our laboratory and findings in patients with nonthyroidal neuromuscular disorders have been reported previously by one of us (Y. T. O.).[3]

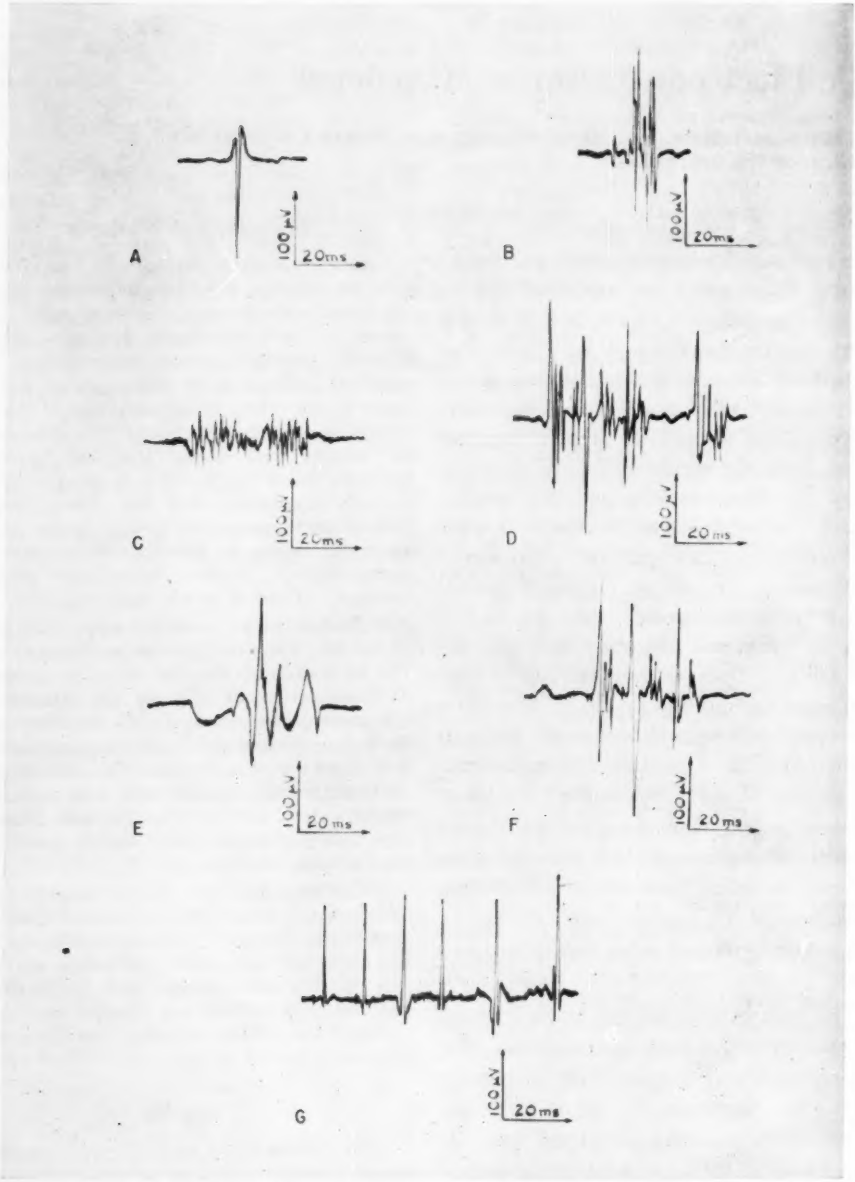
A clinical model electromyograph (Meditron Model 201A) was used with an oscilloscope screen and loudspeaker to monitor the muscle potentials. A monopolar needle electrode was inserted into the muscle to be examined. The indifferent electrode was a metal plate and was placed on the skin directly over the muscle. The usual screened preamplifier and amplifier was used to provide adequate signal gain for the recordings. Magnetic tape and photographs were used to record the muscle-action potentials.

Action potentials were obtained from the biceps brachii and, in some instances, from the quadriceps femoris muscles upon voluntary contraction and relaxation and upon tendon and muscle tap. Several electrode insertions were made in each muscle, and each such insertion was moved to several different areas. At least six reflex stimulations were performed in each patient.

Results

The following four different abnormal findings were observed in electromyograms from patients with myxedema.

A. Polyphasicity.—In normal muscle, on voluntary motion the motor-unit-action potential consists of a single biphasic or triphasic complex (Figure, *A*). Only 2% to 5% of potentials are polyphasic in the normal. In myxedema, polyphasic action poten-



Line drawings reproduced from photographs of muscle potentials. Records are from biceps brachii of various patients. *A*, normal motor-unit action potential of voluntary motion. After completion of the triphasic complex, the tracing returns to a straight baseline ("electrical silence"). *B*, polyphasic action potential on voluntary motion in myxedema. *C*, a series of low-voltage short-duration motor-unit potentials superimposed on base line in myxedema. *D*, hyperirritability in myxedematous muscle. An irregular train of potentials is seen following movement of the needle electrode. *E*, short burst of electrical activity following tendon tap in normal. The slight wave preceding the action potential is mechanically induced by the tap. *F*, prolonged train of activity following tendon tap in myxedema, termed repetitive discharge after reflex motion. The upright wave preceding the first large spike is mechanically induced by the tap. *G*, sustained repetitive action potentials characteristic of myotonia.

tials were found commonly. They showed multiple-peaked complexes of increased duration and voltage (Figure, *B*). In some patients with myxedema as many as 50% of the action potentials were of this form.

B. Low-Voltage Short-Duration Motor Units.—After voluntary motion is completed in the normal muscle, action potentials rapidly disappear and the electromyogram returns to a steady baseline ("electrical silence") (Figure, *A*). In myxedema, irregularly timed potentials of low voltage and of short duration were often found superimposed on the baseline for some time after voluntary motion had ceased (Figure, *C*). These potentials were also seen after cessation of reflex motion. In our normals, low-voltage short-duration potentials are not seen.

C. Hyperirritability.—Normally, movement of the exploring electrode in the muscle elicits a very brief burst of electrical activity. In myxedema, movement of the electrode often resulted in an irregular train of activity, showing potentials of excessive voltage and lasting some three to five times as long as the activity after movement of the electrode in the normal (Figure, *D*). This hyperirritability was also elicited by tapping the muscle with a reflex hammer.

D. Repetitive Discharge After Reflex Motion.—When the related tendon reflex is elicited there normally occurs a short burst of action potentials (Figure, *E*). In myxedema, a single tap of the tendon often resulted in a train of repetitive potentials which was considerably longer than the discharge after reflex motion in the normal (Figure, *F*).

Table 1 presents the pertinent laboratory findings in the 20 patients with myxedema and in the 6 with hyperthyroidism, together with the frequency of the 4 abnormal electromyographic findings described above.

In myxedema (Table 1*A*) polyphasicity, hyperirritability, and repetitive discharge after reflex motion were found with approximately equal frequency. Low-voltage short-duration motor units were found less frequently than the other abnormalities.

TABLE 1.—*Laboratory and Electromyographic Findings in Myxedema and Hyperthyroidism* *

Clinical Data				Laboratory Findings			Electromyographic Findings			
Patient	Diagnosis	Age	Race/ Sex	BMR, %	Cholesterol, Mg./100 Cc.	I^{131} Uptake, %	Polyphasicity	Low-Voltage Short-Duration Motor Units	Hyperirritability	Repetitive Discharge After Reflex Motion
					A. Myxedema					
1	P. Op.	68	W/F	−45	660	2	++	++	++	+
2	P. I.	53	N/F	−41	320	3	0	++	++	++
3	P. I.	46	N/F	−38	438	6	0	++	++	++
4	Idiop.	76	W/F	Too ill	250	Too ill	0	0	++	++
5	Idiop.†	78	W/F	−37	307	Too ill	++	++	0	++
6	P. I.	48	W/F	−35	247	3	++	0	++	++
7	P. Op.	49	N/F	−26	200	6	+	0	0	0
8	Idiop.	73	W/F	−35	330	2	+	++	++	++
9	P. I.	62	W/F	−24	400	0	0	0	0	0
10	Idiop. & leukemia	68	W/M	−2	222	0	++	0	++	++
11	Idiop.	58	W/F	−38	285	1	++	0	++	++
12	P. Op.	58	N/M	−38	387	5	++	0	++	++
13	Idiop.	70	W/M	−20	262	4	++	0	++	++
14	P. I.	54	N/M	−25	235	5	++	0	++	++
15	P. Op.	51	W/F	−21	415	0	0	0	+	0
16	Idiop.†	51	N/F	−35	240	5	++	0	++	+
17	Idiop.†	71	W/F	−32	316	1	0	0	0	0
18	Idiop.	56	W/M	−21	277	5	++	++	++	
19	Idiop.	38	W/F	−30	277	1	++	++	0	++
20	P. Op. & rt. hemiplegia	55	W/F	−34	465	1	R:++ L:++	0	++	++
					B. Hyperthyroidism					
1	Exophthalmic	51	W/F	+25	174	51	0	0	+	0
2	Exophthalmic	40	N/F	+77	98	57	0	+	0	0
3	Exophthalmic	15	N/F	+31	168	91	0	0	0	0
4	Exophthalmic	36	N/F	+64	150	57	0	0	0	+
5	Nonexophthalmic	26	N/F	+43	108	68	0	0	0	0
6	Nonexophthalmic	30	N/F	+89	115	62	0	0	0	0

* P. Op. indicates postoperative myxedema; ++, present consistently; +, present but not consistently; P. I., post-I^{131} myxedema; 0, absent; Idiop., idiopathic myxedema.

† Taking thyroid medication at time of electromyographic examination.

TABLE 2.—*Electromyographic Findings Before and After Treatment of Myxedema* *

Patient	Before Treatment				After Treatment			
	Polyphasicity	Low-Voltage Short-Duration Motor Units	Hyperirritability	Repetitive Discharge After Reflex Motion	Polyphasicity	Low-Voltage Short-Duration Motor Units	Hyperirritability	Repetitive Discharge After Reflex Motion
1	++	++	++	+	++	0	+	+
3	0	++	++	++	0	0	0	0
6	++	0	++	++	0	0	0	0
7	+	0	0	0	0	0	0	0
14	++	0	++	++	0	0	0	+
16	++	0	++	+	++	0	+	0

* ++ indicates present consistently; 0, absent; +, present, but not consistently.

There was no constant relation between findings, no one finding being invariably associated with another. Although it is possible that repetitive discharge after reflex motion is merely another evidence of hyperirritability due to movement of the needle electrode with the reflex, it was found in two patients in whom hyperirritability, as defined, was not present. There appeared to be no direct relation between the severity of the disease and the occurrence of any of these findings. Eighteen of twenty patients showed at least one of the abnormal findings, and sixteen of twenty showed at least two of the abnormalities. Upon treatment, the delayed reflex was found clinically to return to normal in a variable length of time, occasionally before the complete attainment of euthyroidism. Of the six patients reexamined by electromyography after clinical disappearance of the abnormal reflex (Table 2), three showed completely normal electromyograms, two showed improvement toward normal, and one was unchanged.

Three patients with thyrotoxicosis showed no electromyographic abnormalities other than spontaneous motor-unit potentials due to tremor. Each of the other three thyrotoxic patients had an occasional abnormal potential of one of the types described (Table 1*B*).

The electromyogram in myotonia does not resemble that of myxedema. In myotonia, the characteristic finding is the presence of long trains of sustained repetitive motor-unit discharges, often seen after any type of muscle activity (Figure, *G*).

Comment

The cause of abnormal muscle contraction and relaxation in myxedema is unknown. Histologically, there are no significant changes found in human muscles on biopsy.[4] Muscle hypothermia does not appear to be responsible, either in experimental animal [5] or in human [2] myxedema. Although central nervous system manifestations occur in myxedema,[6] the abnormality of the tendon reflex does not appear to reside in the central portion of the reflex arc because the latent period from the tendon tap to the start of muscle contraction is not delayed.[2]

There have been few reports of electromyographic findings in myxedema,[2,5,7-9] and, in general, no characteristic findings have been reported. Lambert and co-workers [2] found no prolonged motor-unit discharge or reflex after-discharge in "most instances." When reflex after-discharge was found, it appeared to bear no constant relation to mechanical events. They concluded, therefore, that abnormal conduction and excitation were not responsible for the abnormal events of contraction. On the other hand, Hoffman and Holmgren [5] found that high-frequency electrical stimulation of muscles of thyroidectomized rats resulted in prolonged contraction and relaxation and, often, repetitive muscle-action potentials.

In the present study, abnormal electromyograms were found frequently in myxedematous persons with delayed tendon reflexes. The incidence of findings in this study may be accounted for by the use of sensitive recording apparatus, the exclusive use of needle electrodes inserted into the mus-

cle instead of surface electrodes, and the restriction of the study to patients with frank disease. Four electromyographic abnormalities were found, none of which, by itself, characterizes myxedema. The combination of two or more of these findings, however, is so unusual in other neuromuscular disorders as to be highly suggestive of myxedema.

The relation of the electromyographic findings to the mechanical events of muscular contraction in myxedema is uncertain. Myotonia is also characterized by abnormal relaxation, and Lambert and Sayre [7] found that the electrical activity of muscles of thyroidectomized rabbits resembled the electrical activity of myotonic muscle. However, myotonia is distinctly different from myxedema mechanically,[2] and the electromyographic findings in humans in this and previous studies [2,8] do not resemble myotonia. Therefore, the abnormal reflex of myxedema cannot be considered due to a myotonia-like change or to true myotonia, although rarely the two may coexist.[9]

There is evidence that the site of action of thyroid hormone on the neuromuscular apparatus is at the level of, or proximal to, the neuromuscular junction.[10-11] Ingold [10] demonstrated in thyroidectomized rats that the prolonged muscle contraction and relaxation time in myxedema can be abolished by blocking transmission at the neuromuscular junction with curare. She postulated that thyroid hormone acts at the junction by regulating the number of muscle fibers discharged with each nerve impulse, excess hormone tending to decrease and lack of hormone tending to increase the number discharged. Since measurements are made from muscles composed of many motor units, repetitive or dyssynchronous discharge or discharge of increased numbers of muscle fibers per unit will tend to prolong the over-all time for contraction and relaxation of the composite muscle. In this regard it is of interest that each of the four electromyographic abnormalities found in myxedema in this study may be considered manifestations of repetitive or dyssynchronous discharge or discharge of increased numbers of muscle fibers. Decreased contraction time occurs in hyperthyroidism.[2] The absence of electromyographic abnormalities of the type described here in patients with severe hyperthyroid myopathy does not contradict the hypothesis that fewer than normal muscle fibers are discharged when excess hormone acts at the neuromuscular junction. The reversal of the electromyographic and mechanical findings reported here, on treatment of the myxedema with dessicated thyroid, is in keeping with this concept, according to which the exogenous hormone would act at the neuromuscular junction to regulate motor unit discharge in the normal synchronous manner, with discharge of normal numbers of fibers, resulting in a normal contraction pattern for the composite muscle.

Summary

The electrical activity of the biceps brachii muscle was studied electromyographically in 20 patients with frank myxedema, each of whom showed the characteristic delayed tendon reflexes of myxedema by clinical examination. Two or more of the following abnormalities were found in 16 of the 20 electromyograms: (*a*) polyphasic action potentials, (*b*) hyperirritability, (*c*) repetitive discharge after reflex motion, (*d*) low-voltage short-duration motor-unit potentials. After treatment of the myxedema, clinical and electromyographic abnormalities reverted toward normal. Six patients with severe thyrotoxic myopathy did not show these electromyographic findings. The possible role of abnormal conduction and excitation in the pathogenesis of the slowed tendon reflexes in myxedema is discussed.

The electromyogram in myxedema has characteristic abnormalities which may be useful in the diagnosis of this disease.

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Case Reports

Double Mitral Valve

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A double mitral valve is one of the rarest of congenital anomalies of the heart. Only 17 instances have been reported. Herein we report the 18th case and the only one in which cardiac catheterization and angiocardiography were performed.

Report of Case

A 55-year-old bartender who drank whiskey freely was first admitted into the Episcopal Hospital on June 3, 1954, for treatment of a fractured mandible sustained during a fist fight while intoxicated.

He was told at the age of 14 years that he had a heart murmur. At the age of 51 he was told that he had hypertension. Since that time he had been conscious of slight shortness of breath after climbing three flights of steps but only when under the influence of liquor.

Physical examination revealed a well-developed man except for possibly disproportionately thin lower extremities. The blood pressure in the right arm was 204/110 mm. of Hg, in the left arm, 170/110 mm. of Hg; in the lower extremities it was frequently unobtainable and when found (rarely) was 160/130 mm. of Hg. Arterial pulsations were visible in the infrascapular regions. The cardiac impulse was focal, 1 cm. outside the left midclavicular line in the fifth intercostal space. Normal sinus rhythm was present. First aortic sound was absent, the second pulmonic sound was slightly accentuated and louder than the second aortic sound. The sounds at the mitral area were of normal intensity. A systolic murmur of moderate pitch was audible over the entire precordium and loudest (Grade 3) in the aortic area. A systolic murmur was also present in the neck and in the left posterior hemithorax. The remainder of the physical examination, including fundoscopy, was normal.

Roentgenologic study of the chest revealed notching of the right fifth to ninth ribs and of the left fifth to eighth ribs. The lungs were normal. The aortic knob, in the anterior projection, was small and irregular in outline. The pulmonary segment was slightly convex. The left lower pole of the heart was displaced to the left and downward. The posterior wall of the esophagus in the region of the superior portion of the descending aorta showed, in the left anterior oblique projection, a smooth anterior indentation that pulsated. The esophagus was not displaced in any projection by the left atrium. There was slight overlapping of the spine by the convex left ventricle. Calcification of the aortic valve was noticed fluoroscopically and confirmed by planigraphy. The conventional roentgenogram of the chest and the planigram demonstrating calcification of the aortic valve have been published elsewhere.[1]

The electrocardiogram showed normal P waves. The P vector was directed 35 degrees laterally. The QRS vector was directed 60 degrees posteriorly. The length of the S wave in Lead V_1 was 44 mm., and that of the R wave in Lead V_6 was 26 mm. The intrinsicoid deflection in Lead V_6 was 0.04 second. The ST-T vector was relatively opposite to that of the QRS. The ST segment was convex upward when depressed, and the T wave was symmetrical. These findings were regarded as consistent with left ventricular hypertrophy and myocardial abnormality.

Simultaneous biplane stereoscopic venous angiocardiography revealed coarctation of the aorta distal to the origin of the left subclavian artery (Fig. 1). The descending aorta distal to the coarctation was opacified and narrowed. The left ventricle was enlarged and its walls thickened. The left atrial volume was 346 ml. (approximately three times normal) and did not vary in size during

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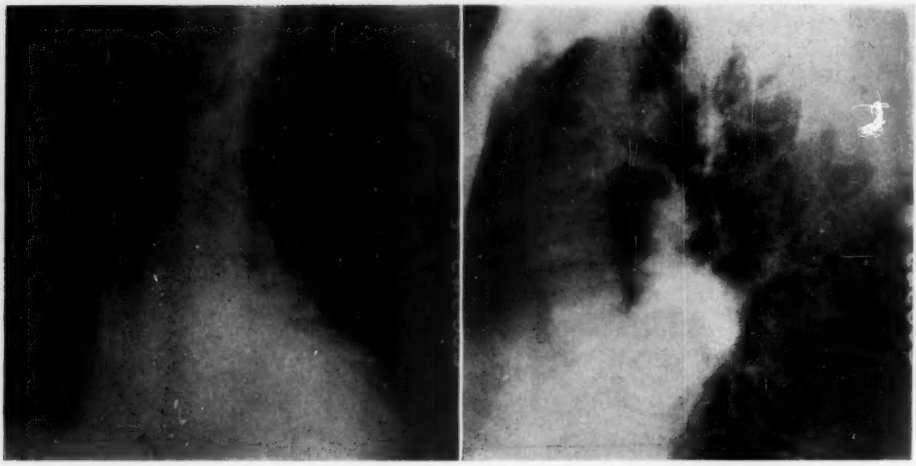

Fig. 1.—Simultaneous venous angiocardiograms in the anterior and lateral projections 11.9 seconds after mitral opacification of the right atrium. Note the enlarged left atrium and left ventricle. The coarctation of the aorta is distinctly visible on the lateral projection immediately distal to the left subclavian artery.

the cardiac cycle. The angiocardiographic circulation times were all prolonged.

The fractured mandible was successfully repaired. He was discharged on June 15, 1954, with a diagnosis of coarctation of the aorta, calcific aortic stenosis, presumably a bicuspid aortic valve, and left ventricular hypertrophy and enlargement.

He was readmitted into the Episcopal Hospital on Nov. 19, 1956, because of shortness of breath which began four months previously with a bout of dyspnea while in bed. Five subsequent attacks of nocturnal orthopnea occurred, with intervening continuous and progressive shortness of breath. Once he collapsed while walking but quickly recovered. One night, while drinking, he suddenly became very short of breath and was brought by the police to the hospital.

Physical examination revealed tachypnea, cyanosis, and pulmonary edema. The heart had increased in size, so that the apical impulse was now in the sixth intercostal space at the left anterior axillary line. After recovery from dyspnea, the blood pressure was 129/80 mm. of Hg in the right arm and 116/78 in the left arm. It was not obtainable in the lower extremities. The femoral artery pulsations were barely palpable. The oscillometric index in the thighs was 2.5 units and in the legs, 0.5 unit. Compared with the previous studies, the chest roentgenogram revealed a slight increase in heart size and the electrocardiogram showed no significant change.

Cardiac catheterization was done. The pressure in the right ventricle was 40-50/0 mm. of Hg and in the pulmonary artery, 35/10 mm. of Hg. The pulmonary artery wedge pressure was 40/10 mm. of Hg.

There was a mild anemia. No abnormality was noticed in the urine. The serological tests for syphilis were negative. Tests of liver function were normal.

The patient was desirous of operative relief, and we agreed that it was needed. The risk, which was very great, was explained to the patient. Not only were two blocks present, but the aortic one was probably due to degenerative changes in a bicuspid valve. It appeared to us and the surgeon (Dr. Thomas J. E. O'Neill) that the significant block was produced by the aortic valve and not by the coarctation because the blood pressure was normal in the upper extremities and adequate collateral circulation was suggested by the rapid opacification of the descending aorta. It was therefore decided to do an aortic valvotomy first.

On Dec. 17, 1956, an aortic valvotomy was done, with use of the Alameida dilator through a left ventricular approach. Shortly thereafter, cardiac arrest occurred. Sinus rhythm was restored following cardiac massage and intracardiac epinephrine. The blood pressure was stabilized at 140/100 mm. of Hg with intravenous arterenol. However, the patient remained unconscious and died two hours after surgery.

Necropsy.—Necropsy examination revealed no abnormal findings other than those of the cardiovascular and urologic systems.

There was a congenital anomaly of the kidney. The lower pole of each kidney was fused across

Fig. 2.—Necropsy specimen of aorta, showing site of coarctation. The ligamentum ductus arteriosus is also shown and is attached to the right branch of the pulmonary artery.

Fig. 3.—Necropsy specimen of calcific bicuspid aortic valve.

the midline of the body, producing a horseshoe-shaped kidney.

The renal blood vessels and ureters were normal.

The ascending aorta was 34 mm. in diameter. The origin of the brachiocephalic vessels was normal. The left subclavian artery was slightly enlarged to 18 mm. in diameter. The aorta, immediately distal to the left subclavian artery, was narrowed to 18 mm. in diameter. It tapered over a distance of 22 mm. to an external diameter of 9 mm. At this site there was a diaphragmatic-like structure occluding the aortic lumen except for a centrally located opening, 1 mm. in diameter. The aorta then increased in size to an external diameter of 23 mm. The wall of the descending aorta immediately distal to the coarctation was thin and showed localized ballooning on pressure. The aortic intima was the seat of atherosclerotic plaques extending for a distance of 5 cm. proximal to the coarctation and 2 cm. distal to it. Immediately proximal to the narrowest portion of the descending aorta was a ligamentum ductus arteriosum, 4 mm. wide and 7 mm. long, extending to the right branch of the pulmonary artery immediately distal to its origin. The coarctation of the aorta is seen in Figure 2.

The heart weighed 575 gm. The left ventricular wall was 17 mm. thick. The right ventricular wall was 3 mm. thick.

Fig. 4.—The double mitral valve, viewed from the left atrium.

Fig. 5.—The double mitral valve, viewed from the left ventricle. A pledget of cotton is seen in the accessory ostium. A wire is inserted in the base of the aortic cusp through the surgical tear.

The aortic valve was bicuspid (Fig. 3). The free edges of the two aortic leaflets (left and right anterior cusps) were thickened and rolled. The leaflets were minimally adherent to each other at their insertion into the aorta. The remainder of the leaflets and the sinuses of Valsalva were filled with large calcific masses. The ventricular surface of the leaflets was smooth but knobby, due to the calcific masses on the aortic surface. The aortic orifice did not admit a finger. An irregular opening, 8 mm. in diameter, the result of surgery, was found in the base of the left anterior aortic cusp. The ostia of the coronary arteries were located slightly above the line of closure of the aortic valve and were patent, as were the coronary arteries.

There were two mitral orifices, the accessory orifice being smaller and anterior to the principal orifice (Figs. 4 and 5). The long axes of these two orifices were in the same plane, separated by a distance of 1 cm., and did not approach each other. The diameter of the principal orifice was 3.5 cm. and that of the accessory one, 1.0 cm. The accessory orifice appeared to be located mainly in the anterior cusp. Separating the accessory from the principal orifice was a bridge of tissue, 1.0 by 1.5 cm., extending from the anterior to the posterior cusp. The chordae tendineae around the principal orifice inserted into an enlarged posterior papillary muscle. The chordae tendineae around the accessory orifice inserted into an enlarged posterior papillary muscle. The chordae tendineae around the accessory orifice inserted mainly on a smaller anterior papillary muscle and partly on the posterior papillary muscle. A lone chorda tendinea extended directly from the leaflet of the accessory orifice to the middle of the interventricular septum. The cusps of each orifice were not thickened and were not opaque to transmitted light.

Comment

A double mitral valve was first reported by Greenfield,[2] who found it in a 26-year-old man who died of acute myelitis. Fourteen cases were collected from the literature by Wimsatt and Lewis in 1948.[3] since then, three additional cases [4-7] and another with mitral valve triplication [8] have been reported.

Wimsatt and Lewis's case occurred in the heart of a yak calf. In their brilliant study, Wimsatt and Lewis could not agree on the embryologic development of this congenital anomaly. Wimsatt thought that it was due to a subdivision of the mitral ostium through an abnormal mediolateral fusion of the mitral cusp primordia, whereas Lewis thought that it was due to retention of the left portion of the common atrioventricular canal, with consequent reduction of the mitral ostium and alignment with it. We cannot resolve this difference of opinion or offer a more plausible explanation.

Although both ostia may be of equal size, the accessory one is usually smaller than the principal one. The smaller ostium is anterior and tends to lie to the right of the larger one, its long axis forming a variable angle with that of the larger one. Their axes tend to converge slightly on the left. Both ostia are guarded by well-defined valve cusps. The ventrally situated ostium is supplied by ventral papillary muscles, and both cusps of the dorsally situated ostium, by the dorsal papillary muscles.[3]

The anomaly has been recognized only at necropsy. No clinical manifestations of this anomaly are recognized even in retrospect except for the report by Davies and Fisher.[9] In their case (a 17-year-old boy with coarctation of the aorta and ruptured intracranial and dissecting aortic aneurysms), they occasionally heard a diastolic or presystolic mitral murmur which they believed may have been produced by the double mitral valve. They did not, like others, believe that any cardiac functional disturbance was produced by this anomaly.

In our patient, left ventricular enlargement and a mitral systolic murmur were present. Angiocardiography demonstrated left atrial enlargement and homogeneous opacification of the left atrium and left ventricle, findings consistent with mitral insufficiency but not pathognomonic of mitral valve disease. These findings were also seen in isolated coarctation of the aorta, in isolated aortic stenosis, and even in primary pulmonary hypertension.[10,11] The pressure curves were also scrutinized for evidence of mitral regurgitation, but none was found. The normal appearance of the mitral curtains likewise suggests no significant functional disturbance. The normal appearance of the valves indicates the benign nature of this anomaly, which is sup-

ported by the fact that all persons with this anomaly have died of causes unrelated to the anomaly and frequently in the sixth, seventh, and eighth decades of life.

The anomaly is usually associated with other congenital anomalies, most commonly bicuspid aortic valve, coarctation of the aorta, and hypoplasia of the aorta. Less commonly, there may be an atrial or ventricular septal defect, a hypoplastic pulmonary artery, or a patent ductus arteriosus.

Except as an accidental operative finding, it appears to us that a diagnosis during life is possible by demonstrating in the left ventricle a double stream of contrast substance during left atrial emptying by means of high-speed or selective angiocardiography.

Summary

The 18th case of a double mitral valve is reported in a man with coarctation of the aorta, calcific stenosis of a bicuspid aortic valve, and a horseshoe-shaped kidney. The double mitral valve produced no recognizable findings or symptoms. It is suggested that by high-speed or selective angiocardiography it might be possible to demonstrate a double left atrioventricular stream of contrast substance.

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Scleroderma Associated with Osteopoikilosis

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Osteopoikilosis, also called "osteopecilia" or "spotted bone disease," is a rare condition of bone, manifest by multiple small islands of dense bone formation in areas where normally cancellous bone is found. Ritvo [1] states that these lesions "vary in size from 2-3 mm. to 3 cm. occurring frequently in the epiphysis and adjacent parts of the metaphysis, usually being more numerous in the latter. The areas of density lie in the cancellous bone, and, as a rule, are entirely free from the cortex. Patients have been observed over a number of years with no apparent change in the roentgen manifestations." While the apparently hereditary, and essentially benign, course of the disease is stressed in most reports,[2-6] a considerable amount of literature has accumulated concerning associated conditions.[7-11] Two types, distinguished roentgenologically, are described: linear and millet-like.

Most of these associated defects are related to skeletal and cutaneous lesions. The condition known as dermatofibrosis lenticularis disseminata, a primarily vesicular but later fibrotic lesion of skin, occurs with greater statistical incidence in patients with osteopoikilosis.[7,8] Other related connective tissue disorders [11] and, indeed, metabolic disturbances,[9] have been encountered. Yet, despite universal recognition of this entity, usually discovered only incidentally on roentgen examination,[3,12] osteopoikilosis has not been definitely linked to another disease manifesting abnormal calcification with cutaneous lesions—scleroderma.

The diagnosis of scleroderma has been made with greater frequency in recent years.[13] Concomitantly, there is developing an increasing awareness of the visceral and metabolic complications of this disease. Calcium metabolism has been repeatedly studied in view of the frequent occurrence of soft-tissue calcifications in scleroderma. This, referred to as the Thibierge-Wiessenbach [14] syndrome, is one manifestation of a systemic calcinosis that is only partially demonstrable on x-ray films of the affected tissues. Yet reports of the radiological findings in cases of scleroderma do not indicate a remarkable incidence of osteopoikilosis in that disease. The finding of calcifications compatible with "spotted bone disease" can, however, be distinguished in several published papers on the subject.[15-17]

In a patient recently observed at the United States Army Hospital, Fort Dix, the conditions of scleroderma and osteopoikilosis were found to exist simultaneously.

Report of Case

A 35-year-old man was admitted to the hospital for acute stiffness and swelling of the small joints of the hand of one week's duration. He also complained of chronic tightening of his skin accompanied by migratory arthralgias for the past six years. The patient had in 1940 developed intolerance to cold, manifested by cyanosis, followed by blanching and pain of his finger tips when exposed to cold. This had persisted to the winter of admission. In addition, the joints of his hands, elbows, knees, and especially of his shoulders had become quite painful. At times there was heat, swelling, and pain, causing marked limitation of motion of his wrists and fingers. In the past two years his lips had become severely cracked and fissured even in warm weather, and a puckered appearance of his face had also been noticed. The admitting complaints were not only of acute swelling and pain of the distal and proximal interphalangeal joints, but also of a feeling of tightness in upper chest and shoulder girdle.

In 1949, for his joint difficulties, he was evacuated from Japan to Percy Jones Army Hospital, Battle

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Creek, Mich., where he was treated by "white pills," and a biopsy of his skin was done. At that time he was told to remain in contact with medical facilities because of a suspicious finding on biopsy, and he was discharged. He reenlisted in the spring of 1956 and was able to pass physical examinations at induction.

Prior to this enlistment the patient did manual labor for several years, experiencing diminished physical vigor only recently. There was no history of familial disease. Systemic review was negative as to cardiovascular difficulties. The patient did, however, describe a sense of "pressure" or "mass in the chest" on leaning forward. There was no true dysphagia, nor did food seem to stick in his chest. Yet bulky foods would occasion distress immediately after swallowing. There was no history of abdominal cramping, bulky stools, or diarrhea.

Physical examination revealed a well-developed white man in acute distress from joint pains and aching muscles. Findings on examination of ear, nose, and throat: negative. Neck: pain on any degree of cervical motion, severe muscle pain, thyroid not felt. Chest cage revealed marked kyphoscoliosis. The lungs were clear to percussion, but the breath sounds were uniformly diminished. Heart: normal. Abdomen: no organs or masses felt. Genitorectal examination: negative. The skin was hard and drawn, appearing almost pearly white, with a leathery texture of the entire hand surface and over the face (Fig. 1*A*). There seemed to be little subcutaneous tissue. The dorsum of both hands was mainly devoid of hair, and over the dorsum of the left hand there was a large area of melanin pigmentation (Fig. 1*B*). The remainder of the skin was quite dry and ichthyotic. The upper extremities showed mild ulnar deviation of both hands, with acute arthritis of the proximal and distal interphalangeal joints. The knees and ankles were not involved. There was marked inability to raise either arm above 90% at the shoulder joint, with marked pain at that point.

Laboratory Studies.—The initial WBC was 12,000, with 72% polymorphonuclear leukocytes and 28% lymphocytes; Hgb., 14.4 gm. per 100 cc.; E. S. R. (Wintrobe), 24; hematocrit, 45%. Urine: specific gravity, 1.028; negative for albumin, sugar, and acetone; no sediment. BUN: 16 mg. per 100 cc. Two-hour postprandial blood sugar: 96 mg. per 100 cc. Urinary concentration test: 1.032 maximally. P. S. P. showed 90% excretion of dye in one hour. Cephalin flocculation: 2+ in 48 hours. Thymol turbidity: 1 unit. Sulfobromopathalein: 2.5% retention of dye in 45 minutes. The C-reactive protein was persistently elevated. The urine showed positive reducing substance with use of acetylsalicylic acid therapy. Serology was negative (cardiolipin flocculation). Urinary Sulkowitch test: 1+. Serum calcium, 9.1 mg. per 100 cc.; phosphorus, 3.9 mg. per 100 cc., and alkaline phosphatase, 2.3 Bodansky units. While taking a six-day Bauer-Aub diet the patient excreted 490 mg. of urinary calcium in 72 hours (72-hour intake, 390 mg.). Cold agglutinins, cryoglobulins, and Streptococcus MG agglutinins were not demonstrated. Urinary 17-ketosteroids were within normal limits. The latex fixation test for rheumatoid arthritis (Dr. J. Singer) was negative. Electrocardiograms were within normal limits. Serum electrophoresis showed a total protein of 7.3 gm. per 100 cc., fractioned into the following components: albumin, 53.8% α_1-globulins, 2.9%;

Fig. 1.—*A*, the facies of the patient, showing marked tightening of skin about the mouth and eyes. *B*, the hands, characteristic of scleroderma, showing hard, pearly skin. There is loss of hair over the dorsum of the hand. Note the large pigmented lesion on the dorsum of the left hand. Early sclerodactylia is noted.

Fig. 2.—The right hand of the patient. In addition to the soft-tissue calcifications near the second phalanx of the index finger and the interphalangeal joint of the thumb, there are minute lesions of osteopoikilosis at the base of the first phalanges of the second, third, and fourth fingers. The other bones are similarly affected. Early bone resorption of the terminal phalanges is evident.

α_2-globulins, 12.4%; β-globulins, 12.4%; γ-globulins, 14.5%, and lipoproteins, 4.0%. This would indicate an elevated α_2-globulin value by this method (paper electrophoresis, method of First Army Area Medical Laboratory).

X-ray examination of the chest revealed moderate scoliosis of the thoracic spine, counterclockwise rotation of the heart, and minimal and early fibrosis of the lungs.

Films of knees, cervical region, thoracic region, lumbar spine, pelvis, and long as well as small bones showed evidence of discrete patches of osteopoikilosis in the epiphyseal ends of all bones (Figs. 2 and 3). These were dense areas of intraosseous calcification, 50 mm. to 1 cm. in size, and were random, rather than linear, in distribution. Hand x-rays revealed soft-tissue calcifications lateral to the middle phalanx of the index finger of the right hand, as well as at the interphalangeal joint of the thumb. In addition, minimal areas of osteopoikilosis were evident in the other small bones of the hand.

Findings on barium enema were reported as negative. A barium swallow noted slight narrowing of the lumen of the esophagus, with moderate straightening of the normal curve of the esophagus, compatible with the diagnosis of scleroderma. Upper gastrointestinal and small-bowel series were negative.

Three biopsies were done of the skin of the dorsum of the left hand. The first was reported as "normal skin." A second was reported as "normal maturation of epidermis, fragmentation and separation of collagen, as well as condensation of collagen into thick bundles." These changes were not considered specific. Findings on the last biopsy were reported: "Epidermis shows normal maturation with prominent granular cell layer and thick keratin layer. The dermis showed slight coarsening and fragmentation of collagen, but no significant degeneration is present. There is some blood vessel compression. If the biopsy was from the dorsum in an area normally hair bearing, the changes are consistent with early scleroderma." (Capt. Ray D. Reed [MC].)

The patient was given a regimen of bed rest and salicylate therapy of 7.2 gm. per day. His acute joint pains subsided rapidly on this regimen, and physical therapy was instituted. He was at no time febrile, yet the C-reactive protein was repeatedly positive and the sedimentation rate remained elevated. With physical therapy and acetylsalicylic acid alone, the patient's performance on an "Activities of Daily Living Test" was improved 60%. Unfortunately x-rays during previous hospitalization were unavailable. The biopsy done in 1949 and reported by the Armed Forces Institute of Pathology was nondiagnostic of scleroderma, although minimal changes led this agency to request follow-up studies.

One month after admission the patient was symptom-free of acute arthritis and suffered only from the tightening of the skin of hands and face. He was presented to a medical board for consideration of separation from service.

Comment

The above patient, in addition to manifesting the classical lesions of scleroderma clinically, had areas of soft-tissue calcifications in his right hand (near the proximal interphalangeal joint of the second finger and the interphalangeal joint of the thumb). At the epiphyseal and adjacent metaphyseal ends of all bones, large and small, the lesions of osteopoikilosis were readily discerned. It was difficult to relate the manifestations of intra- and extraskeletal calcifications in concurrence of scleroderma and osteopoikithe patient without speculation as to this losis.

That patients with scleroderma have an associated disturbance of calcium metabolism has long been suspected.[14] The incidence of calcinosis in such patients has been estimated as 10%.[13] However, careful calcium-balance studies are not too numerous. The

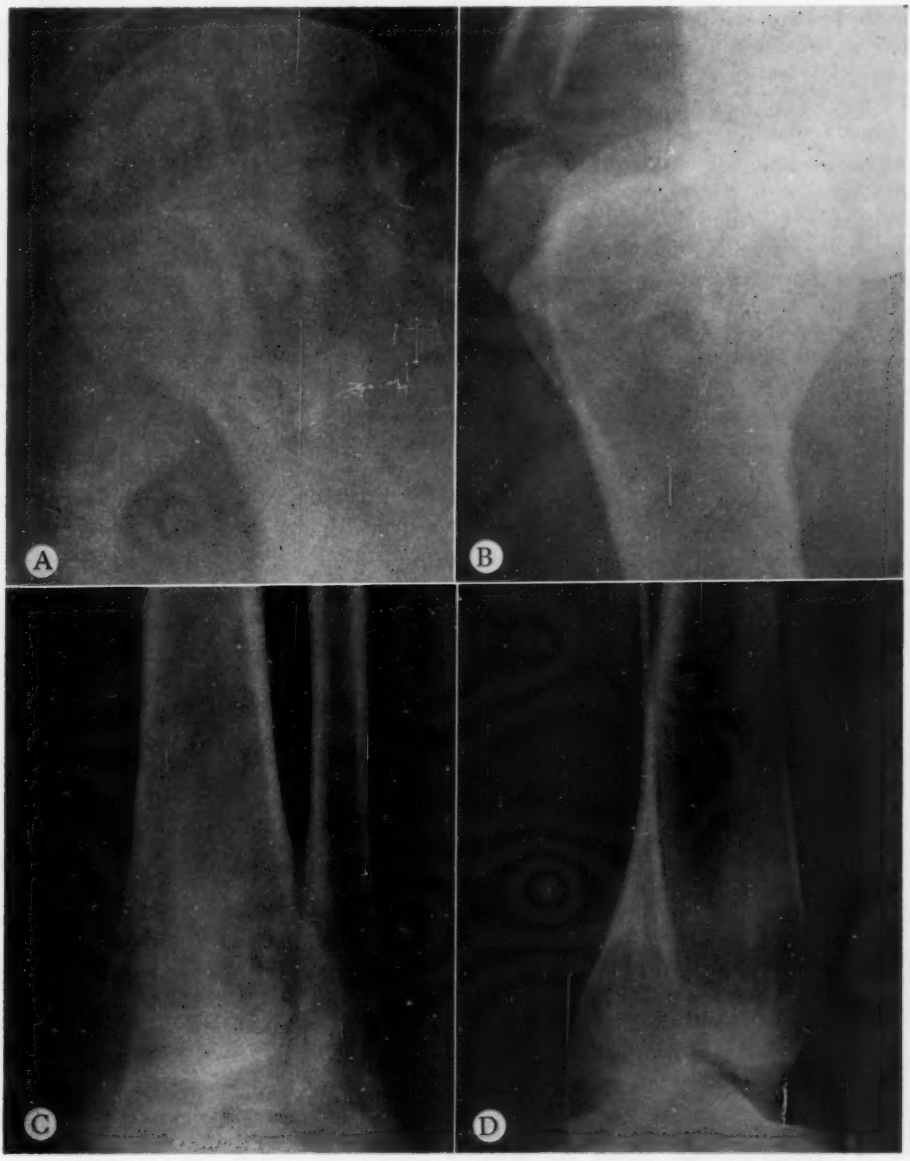

Fig. 3.—*A*, left humerus of patient with scleroderma. The lesions of osteopoikilosis are seen. Similar lesions were evident in all bones. *B*, right humerus in the same patient. *C*, left tibia and fibula, showing osteopoikilosis. *D*, right tibia and fibula, showing osteopoikilosis.

skin of patients with scleroderma has been shown to contain calcium to a greater degree than that of normal control subjects. This calcium is deposited diffusely in the corium among normal collagen fibers.[18] Klein and Harris,[19] while treating the generalized calcinosis of scleroderma by chelation, were able to effect a remarkable increase of calcium excretion after initially normal values. Diminished calcium excretion in scleroderma is reported by Cornbleet and Struck,[18] who found these patients to

be in positive calcium balance. The case here reported was in negative calcium balance while at relative bed rest, excreting 490 mg. Ca per 72 hours with a 390 mg. per 72 hours intake.

The parathyroids have been repeatedly associated with scleroderma, and, indeed, their surgical removal has been attended with reported relief of scleroderma symptoms.[20] Selye was able to produce sclerodermatous lesions in rabbits by the intraperitoneal injection of parathyroid hormone,[21] and Leriche [22] demonstrated increased skin calcium in these animals. Whether such a pathogenetic mechanism exists in the production of the intraskeletal calcifications of osteopoikilosis is, unfortunately, an unanswerable question as yet. No disorder of calcium metabolism has been associated with this condition, and what few laboratory determinations have been reported have all been within the normal range.

The essentially benign and unchanging clinical course of osteopoikilosis has been mentioned before.[23] Yet Schmorl [24] cited autopsy findings in one patient who had concurrent amyloid disease, and recently Appelman and Moehlig [9] reported two cases of an associated "metabolic craniopathy." Their two cases, mother and daughter, had, in addition to osteopoikilosis, skeletal lesions in the skull sufficient to produce neurological and hypothalamic disturbances. But there is little in the pathology of the bone lesion itself to implicate an acute process: "There is a bone island . . . of spongy trabeculae having lamellar structure, but no Haversian canals. A dense mass of osteoclasts and osteoblasts may form at the island's center. At the periphery, the islands merge into surrounding cancellous bone." [25] This does not correlate well with the concept of an essentially arteritic change that has been postulated in early scleroderma. Indeed, most observers who have studied the lesions are convinced that they are present from youth.[27,28] The diagnosis has been made as early as the seventh month of life.[25] It may be that the increased tendency to calcification, if present at the time of early bone formation, manifests itself first as osteopoikilosis and only later as the calcinosis of scleroderma. Only a long-range study of both diseases, with associated calcium balance studies, could test this hypothesis.

Summary

A case of scleroderma in a 35-year-old white soldier is presented. In addition to soft-tissue calcifications associated with that disease he manifested the lesions of osteopoikilosis.

Osteopoikilosis was present in practically all bones of the patient's body. This consisted of multiple dense areas of bone formation at the epiphyseal and adjacent metaphyseal ends of normally cancellous bone.

The relationship of osteopoikilosis to the calcinosis of scleroderma is discussed.

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Reviews in Internal Medicine

The Laboratory Diagnosis of Pancreatic Disease

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The diagnosis of pancreatic disease is gradually improving, largely as a result of increasing experience in correlating laboratory data reflecting normal and altered physiologic function and newer techniques for assessing the digestive capacity of pancreatic enzymes. Characteristic alterations of the quantity and constituents of the duodenal content have been established. These refinements have emphasized the importance of adequate understanding of the type, mechanism, and application of pancreatic function tests. In the present discussion these procedures are classified with respect to the moiety analyzed or the specific function evaluated (Table 1).

TABLE 1.—*Pancreatic Function Tests*

I.	Determinations of blood or serum constituents
	A. Serum amylase
	B. Serum lipase
	C. Plasma antithrombin titer
	D. Provocative pancreatic enzyme tests
	E. Glucose-tolerance test
	F. Serum calcium
	G. Serum carotene
II.	Determinations of urine constituents
	A. Urinary amylase
	B. Urinary lipase
III.	Determinations of stool constituents
	A. Microscopic examination
	B. Quantitative fecal fat content
	C. Fecal nitrogen content
	D. Fecal trypsin activity
	E. I^{131}-labeled protein meals
IV.	Detection of alteration in digestive capacity
	A. Vitamin A tolerance
	B. Starch tolerance
	C. I^{131}-labeled fat meals
	D. Estimation of phospholipid synthesis with radioactive phosphorus
V.	Detection of abnormalities of pancreatic secretion via duodenal intubation
	A. Volume of secretion
	B. Bicarbonate concentration
	C. Enzyme concentration
	D. Concentration of biliary pigment
	E. Cytology of duodenal aspiration

Determinations of Blood or Serum Constituents

A. Serum Amylase.—Since Elman and his associates,[9] in 1929, confirmed the presence of an increased serum amylase activity in inflammation or obstruction of the pancreatic duct, this analysis has become the most widely applied test of pancreatic disease. Although several biochemical systems have been employed, the saccharogenic method of Somogyi [10,11] is most frequently utilized. Modifications and simplifications now permit accurate estimation of serum amylase activity in less than 30 minutes.[12,13] The normal range for serum amylase by the Somogyi method is 60 to 200 units. The unit is that amount of amylase required to digest 1.5 gm. of starch in eight minutes at 37 C. This enzyme originates primarily in the pancreas; however, smaller amounts may be derived from the salivary glands, liver,[14] muscle,[15] and adipose tissue.[16] This capacity of tissues other than the pancreas to produce amylase has been observed in the restoration of normal serum levels following partial or complete pancreatectomy.[17,18] Amylase in the serum is dependent upon the kidney for excretion. The rate of removal was thought to parallel the glomerular filtration rate,[19] but recent studies indicate poor correlation with the urea clearance.[20]

The amylase levels in acute pancreatitis rise rapidly, attaining a peak in about 24 hours; they return to normal range in 3 to 5 days. The magnitude of the elevation is usually 4 to 6 times the normal value; however, activity as great as 40 times the upper limit has been recorded.[2] In some instances of mild pancreatitis, hyperamylasemia is

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only detectable for a few hours. It is imperative, therefore, that determinations be performed early in the course of the illness. Increased serum amylase activity may persist for longer periods when circulatory collapse and decreased renal blood flow complicate the acute necrosis of the pancreas. The local mechanism responsible for increased release of the enzyme is thought to be increased acinar cell permeability[21] and elevated pancreatic intraductal pressure with retrograde diffusion.[22] The diagnostic implications of this analysis in acute pancreatitis and exacerbations of chronic relapsing pancreatitis are well authenticated; but serial determinations also may demonstrate significant transient elevations in carcinoma of the pancreas.[23-25] Persistently low serum amylase values have been demonstrated in advanced pancreatic insufficiency, resulting either from acute massive destruction[26,27] or from progressive replacement with fibrous tissue.[28-30]

Although elevation of serum amylase is relatively specific, increases may occur in the absence of primary pancreatic disease. Disease of the biliary tract and ampullary sphincter spasm (particularly with certain medications) have produced increased levels of amylase.[31-38] Similar rises have been noted in mumps[39,40]; however, symptoms suggestive of coexistent pancreatitis occur in less than 20% of cases,[41] and the presence of other alterations (lipase elevation) must be ascertained to confirm the existence of the complication.[42] Abnormally high serum amylase activity has been recorded in penetrating[25,43] and perforated peptic ulcer[44-46] and in some instances of intestinal obstruction.[47,48] Increased production or absorption of enzyme is not necessarily the only factor responsible for elevation of serum amylase. As mentioned previously, renal integrity must be maintained for regular elimination of the ferment, and activity as high as 1000 Somogyi units has been reported with renal insufficiency.[49] Notwithstanding the occasional ocurrence of these "false positive" situations, the determination of serum amylase activity is paramount, both early and frequently, in the course of pancreatic disease.

B. Serum Lipase.—Cherry and Crandall, in 1932,[50] demonstrated the presence of a specific fat-splitting enzyme in the serum of animals with pancreatic injury induced by pancreatic-duct ligation. This lipase was demonstrated most abundantly in the pancreas and pancreatic juice, with smaller amounts in the intestinal mucosa, liver, and spleen; esterases, on the other hand, originate primarily in the liver and have the capacity to split esters of lower fatty acids. The selection of a proper substrate is all-important in the specificity of pancreatic lipase. Structural characteristics of the substrate, such as the acyl chain length and the number of ester groups, are critical in eliminating misleading esterase activity.[51] Cherry and Crandall substituted olive oil, a specific lipase substrate, for ethyl butyrate in Loevenhart's method[52] for serum esterase. This method, with few modifications, remains the most reliable and most frequently employed determination of serum lipase. Serum activity is expressed as the number of milliliters of N/20 sodium hydroxide required to titrate the lipase-liberated fatty acids to a phenolphthalein end-point (pH 10.65). The upper limit of the normal range in the serum is 1.0 units. The major deterrent to this determination has been the 24-hour incubation of serum and substrate required prior to titration. A recent modification permits accurate estimation of serum lipase activity with triolein substrate in four hours.[53] Other methods, with use of tributyrin[54,55] and betanaphthyllaurate[56] as substrates, have been suggested, but serious objection to these modifications has been raised on the basis of questionable lipase and esterase specificity.[51,53]

Serum lipase, like amylase, depends upon alterations within the pancreas for increased activity (i. e., increased ductal pressure and cellular permeability) and renal integrity for elimination; however, the increase in acute pancreatitis persists for a somewhat

longer period (7 to 10 days) than the starch-splitting enzyme.[57-59] Some observations have suggested serial lipase determinations as a valuable aid in the diagnosis of pancreatic carcinoma.[58,60] In one series, of 30 cases, elevations in lipase were noted in more than 50%. Organ specificity may be somewhat more certain, but "false positives" have been reported in hepatic and biliary tract disease,[57,61] intestinal obstruction,[48,62] and following the use of opiates.[37,63,64] Increased proficiency, simplicity, and improved technique justify wider clinical application of this test.

C. Plasma Antithrombin Titer.—Comparable quantities of trypsin or trypsin precursor are undoubtedly liberated into the blood with other pancreatic enzymes in various diseases, but no suitable direct analysis has been developed. The hypothetical explanation for this failure is the presence of potent antitryptic substances. Alteration of blood coagulability in various pancreatic disorders attributable to altered tryptic activity is well recognized, and experimental studies have shown that trypsin intravenously produces intravascular clotting and subsequent decrease in plasma fibrinogen and prothrombin content in animals.[65] This effect results from the accelerated conversion of prothrombin to thrombin, and it is not inhibited by heparin. Further investigation has demonstrated proteolysis of prothrombin, fibrinogen, AC-globulin, antifibrinolysin, and antithrombin following intravenous injection of trypsin.[66] Apparently all of these substances return to normal within a few hours with the exception of antithrombin. Indications are that the decreased plasma antithrombin titer, initially induced by a trypsin load, is followed by a compensatory increase persisting for several days[67]; however, additional clarification of the trypsin antithrombin relationship would seem appropriate. Is a threshold range of trypsin release present which maintains normal antithrombin levels? What is the time required per unit amount of trypsin to produce elevation, and is the response quantitatively related? Would elevation or depression of antithrombin occur with a sustained trypsin source? Elucidation of the specific site or sites and mechanism of this phenomenon would be advantageous in the interpretation of results.

Innerfield and his associates applied a modification of the Quick method[68] for determination of antithrombin titer in various pancreatic and related disease states, unrelated conditions and normal controls.[67,69-74] The unit of antithrombin was stated as that amount present in 0.2 cc. of plasma when combined with 0.1 cc. of standardized thrombin which will form a clot in 15 seconds. The normal range of antithrombin activity is expressed as 1 to 6 units. Determinations compare unknowns with simultaneous controls after incubation of 1, 5, 10, and 15 minutes in a water bath at 37 C. An unknown is positive when it requires 100% the control time at 5 minutes and at least 300 seconds at 15 minutes to form detectable clotting.

By the above criteria the authors reported positive results in 90% of 55 cases of acute pancreatitis. Elevations or positive values were obtained in 48% of 23 jaundiced patients with carcinoma of the pancreas. Increased plasma antithrombin activity was demonstrable with acute exacerbations of chronic relapsing pancreatitis, in cystic fibrosis, and with pancreatic cysts. Low antithrombin levels were thought to be significant in certain instances of pancreatic acinar-cell depletion. Specificity, ease of measurement, and relatively prolonged elevation in acute pancreatitis were indicated as the advantages of this determination. Moseley has confirmed the diagnostic value of this procedure in a somewhat smaller series of acute pancreatitis (85% positive results in 18 cases), but he reports less accuracy in carcinoma of the pancreas.[75,76] Several investigators have been unable to verify the specificity of the increased plasma antithrombin titer to pancreatic disease, particularly the high percentage of positiv-

ity in acute pancreatitis.[77-80] This apparent discrepancy may be related to varied technique. Nevertheless, further observation and clarification of the trypsin-antithrombin relationship are needed before the accuracy and applicability of this determination as a pancreatic function test can be evaluated finally.

D. Provocative Pancreatic Enzyme Tests.

The principal of the provocative pancreatic test is to invoke through a specific stimulus a characteristic determinable response in a diseased pancreas that would not occur in a normal organ. Theoretically, two patterns of diagnostic importance may be achieved. The stimulus should produce elevation of serum enzyme in a diseased gland but not in the normal; for example, increased intraductal volume, and thereby pressure, peripheral to blocked ducts. The second possibility is the production of elevated enzyme levels in the normal person, dependent on normal acinar-cell integrity, which would not occur if destruction had taken place, as in pancreatic fibrosis and insufficiency. Some of the discrepancy in the clinical correlation of these tests may lie in the relatively frequent coexistence of duct obstruction and acinar-cell destruction, the degree of enzyme partition alteration provoked by inflammation, the anatomical arrangement and functional capacity of the ampullary sphincter, and the individual response and reaction to calculated dosage of the particular pharmacologic stimulus.

The drugs employed may be classified into three groups, dependent upon their specific site of action. The parasympathomimetics (neostigmine [Prostigmin], bethanechol [Urecholine], and methacholine [Mecholyl]) are applied as acinar-cell stimuli. Secretin, on the other hand, is known to increase the volume and bicarbonate content of pancreatic juice, presumably from the ductile epithelium, without directly influencing enzyme formation. The third group, the opiates, especially morphine, cause ampullary sphincter spasm. These pharmaceuticals have been evaluated, both individually and in combination, as to their ability to induce alterations of serum amylase and lipase content. Knight and his associates[81] estimated serial serum diastase (amylase) activity following neostigmine administration and noted some correlation of response with the type and degree of pancreatic disease. These results were confirmed by Malinowski.[82] Sachar and co-workers[49] were unable to elicit consistency with neostigmine alone or in combination with morphine or methacholine. Wirts and Snape,[84] utilizing both serum amylase and lipase determinations, induced elevations in normal persons with separate injections of methacholine and bethanechol, but little diagnostic correlation was achieved in pancreatic disease. Other investigators[36,83-87] have employed secretin as a provocative agent, but the only satisfactory results were obtained in isolated instances of duct obstruction. Dreiling and Richman[88] subsequently evaluated individual and various combinations of all of these stimuli in a well-controlled study. Although a combination of methacholine, secretin, and morphine evoked the greatest response, only one-half of the controls exhibited elevations of serum amylase; positive reactions were demonstrable in only one-fourth of the patients with pancreatitis. This led to the conclusion that in no instance were patients with pancreatic disease separable from normal persons. Still other provocative tests have been performed in an attempt to utilize changes in blood coagulation as a method for estimation of increased release of pancreatic trypsin. Innerfield[74] compared serum antithrombin titers before and one hour after neostigmine administration and assigned considerable value to the results. A pronounced elevation of titer was regarded as "prima facie" indication of intrinsic pancreatic inflammation, but no data were presented. Shingleton et al.,[89] employing secretin and bethanechol, estimated the alleged trypsin enhancement of blood coagulation by titration against an anticoagulant, Paritol C (sodium polyanhydromannuronic acid sulfate). Positive tests were noted

with pancreatic carcinoma and chronic pancreatitis in a relatively small series. Further evaluation and confirmation of this procedure have not been published as yet.

Serum provocative pancreatic enzyme tests, with occasional exceptions, have proved disappointing in specific diagnosis. This relatively convenient approach and fairly sound principle may be applied more effectively in the future when more specific serum enzymes are identified.

E. Glucose-Tolerance Test.—Glycosuria and hyperglycemia occur relatively often in pancreatic disease. It has been demonstrated that removal of 90% of functioning islet-cell tissue is necessary to induce diabetes mellitus in experimental animals. Whether or not clinical diabetes mellitus precludes extensive destruction remains enigmatic; however, greater or smaller degrees of impairment of endocrine function may result in alteration of carbohydrate metabolism as demonstrated by the glucose-tolerance test. Warren and his associates [90] confirmed this fact and demonstrated its frequency in acute pancreatitis; they concluded that this facet should be evaluated thoroughly in all patients with acute necrosis. Althausen and Uyeyama [91] considered these responses in persons with blood sugar levels of 200 mg. per 100 cc. or higher within two hours after oral glucose administration as clinical or latent diabetics, dependent on fasting blood glucose values of greater or less than 100 mg. per 100 cc.

Maimon and co-authors [92] noted a "diabetic-type" response in eight of nine instances of chronic recurrent pancreatitis. Abnormalities of glucose tolerance were recorded in six of eight determinations in the series of Comfort and associates.[93] King,[94] in a review of the literature, concluded that substantiated diabetes mellitus was present in 35% to 50% of patients with chronic relapsing pancreatitis. The majority of these cases demonstrated either steatorrhea or pancreatic calcification or both, indications of advanced disease; more frequent determinations in earlier suspected cases may indicate impairment of diagnostic significance.

Alteration of dextrose utilization is not uncommon in carcinoma of the pancreas. Again, comparatively few determinations are recorded, so that the incidence is difficult to determine. Berk,[95] in an extensive review of pancreatic neoplasms, noted impaired glucose tolerance in 34 of 163 reported tests, derived from a total of 1449 cases. In his own series, of 33 patients, only 9 were tested and 7 were abnormal. In the group of 88 reported by Knight and Muether,[96] 11 determinations revealed 7 altered curves. Dashiell and Palmer [97] noted a "diabetic-type" response in 18 of 21 instances of carcinomatous involvement. Other authors have emphasized the efficacy of this determination,[98-100] and yet it seems not to have been utilized sufficiently; the results warrant its application in every suspected pancreatic disorder.

F. Serum Calcium.—The significance of the lowered serum calcium in acute pancreatitis was emphasized by Edmonson and Berne,[101] who, in a small series, found values below 7.0 mg. per 100 cc. associated with extensive necrosis of the pancreas and a fatal prognosis. A subsequent report [102] noted hypocalcemia below 9.0 mg. per 100 cc. in 19 of 27 patients with acute pancreatitis. This change became manifest between the first and ninth days, with the lowest levels recorded on the second to fourth days of the disease. The loss of calcium is attributed to the formation of soaps with liberated fatty acids, to the excretion of calcium into the bowel, and to the aspiration of the ion via intestinal and gastric intubation. Determination of the serum calcium may afford a rough guide as to the extent of destruction of the pancreas and, in certain instances, may lend diagnostic support when the serum amylase has returned to normal levels.

Hypocalcemia may occur in an occasional case of chronic recurrent pancreatitis or neoplasm. This defect would depend upon diminished absorption through inadequate

intraluminal digestion of fat or to a relatively greater depletion of protein-bound serum calcium due to concomitant hypoproteinemia. Hypocalcemia has little, if any, diagnostic usefulness per se in the diagnosis of chronic pancreatic disorders.

G. Serum Carotene.—The concentration of the fat-soluble vitamin, carotene, in the body depends upon dietary intake and fat absorption. The plasma carotene concentration, as determined by a modified method of Kimble,[103] correlates closely with a 24-hour fecal fat determinations.[104] Blood carotene is of value as a screening test for various malabsorptive states.[105] In pancreatic disease with blockage of major ducts or extensive acinar-cell destruction, digestion of fat is impaired.[106] Although frank steatorrhea is usually a late sequel of chronic recurrent pancreatitis or carcinoma, moderate depletion of plasma carotene below the normal range of 60μg. to 240μg. per 100 cc. may indicate smaller degrees of defective digestion. When fat absorption is impaired, either through deficient enzymatic activity or affectations of the small bowel or its lymphatics, gradual depletion of carotene stores occurs and the plasma carotene content decreases. This procedure provides a simplified screening device for fat absorption; but it is not, of course, specific for impairment of pancreatic function.

Recently, Kowlessar and McEvoy [107] have recorded the serum desoxyribonuclease I activity in experimental and clinical pancreatic disorders. This depolymerase of desoxyribunucleic acid has been demonstrated in relatively high concentration in the pancreatic tissue of experimental animals.[108] With use of a modification of Allfrey and Mirsky's method utilizing sodium desoxyribonucleate as a substrate, serum desoxyribonuclease I activity was determined. The significant result was a fivefold increase for as long as eight days in the serum of 10 patients with surgical or autopsy-sustantiated acute hemorrhagic necrosis. Further confirmation of these results will be needed before evaluating the procedure; however, it is possible that still another serum pancreatic function test may be available.

Determinations of Urine Constituents

A. Urinary Amylase.—As previously stated, amylase activity in the urine, dependent upon unimpaired renal excretion, parallels serum levels.[19,20] Although isolated specimens have been employed in diagnosis,[109] apparently greater accuracy is derived from determination of 24-hour collections. When the saccharogenic method of Somogyi is utilized for estimation, a corrective factor is introduced for other urinary reducing substances.[110] There is evidence that undetected transient elevations of serum amylase may be detected by this procedure. An additional advantage is the somewhat prolonged period of elevation, 7 to 10 days, with a given episode of acute necrosis. Some studies indicates that 2-hour collection periods are as accurate as 24 hours, allowing more immediate evaluation in an acute emergency.[111,112]

B. Urinary Lipase.—Little emphasis had been placed on urinary lipase content until the recent studies of Nothman and associates.[113,114] This enzymatic activity is attributed to pancreatic liberation of specific fat-splitting substance on the basis of animal experimentation. Pancreatic stimulation increased the activity, whereas pancreatectomy resulted in immediate disappearance. Pancreatic-duct ligation induced marked initial increase, with gradual decrease to very low levels of lipolysis. This initial enhancement evoked by artificial blockage disappeared entirely by pancreatectomy. With utilization of the triolein substrate method of Cherry and Crandall,[50] tests were applied to the urine of 100 normal humans. Average activity of 0.35 and a range of 0.1 to 0.75 units were recorded. Serial determinations on the same and consecutive days did not vary appreciably. The values were uninfluenced by food intake. The clinical application of this analysis in pancreatic disorders is yet to be established;

however, the preliminary data are promising.

Determinations of Stool Constituents

A. Microscopic Examination.—Enzymatic deficiency may be detected by the microscopic identification of undigested food particles in feces. Creatorrhea indicates an abundant number of muscle fibers with typical cross striations in the stool. This variant, particularly in patients ingesting only average amounts of meat, may signify diminished production of trypsin by the pancreas. The specificity of assignment of muscle fibers in the stool to trypsin deficiency is subject to proteolysis by colon bacteria and intestinal motility and transit time.[115]

Steatorrhea may occur in advanced pancreatic insufficiency; theoretically, this excess fat is in the neutral form. Microscopic examination of material stained with Sudan III allows differentiation of various fat moieties. Neutral fat is identified as flakes or droplets with a bright-red-staining quality, while fatty acids appear as orange flakes or needlelike crystals. Various soaps remain colorless unless specifically hydrolyzed to fatty acids by the addition of acetic acid.

The microscopic detection of starch granules is of little diagnostic significance.

B. Quantitative Fecal Fat Content.—The fat content of the feces usually is expressed in percentage of wet or dry weight of the total quantity excreted in a calculated period of time. Some difference of opinion exists as to the reliability of determinations based on dry weight assays; several investigators[116,117] suggest that dessication introduces error through evaporation of volatile fatty acids and conversion of neutral fat to fatty acids. Collection periods have been indicated by inert dyes (such as carmine and charcoal) or by observing representative time relationships, varying from 24 to 72 hours; the latter time interval is more effective in eliminating errors introduced by intestinal transit inconsistencies. The fat intake is ascertained by precalculated dietary content[115,117] or balance studies allowing some variation in amount but accurate recording per meal.[118] The range of intake should approximate 50 to 100 gm. per 24 hours to ascertain maximal absorptive capacity and avoid overloading error. This computation is recorded as per cent excreted or absorbed. Unimpaired fat digestion and absorption is indicated when 5% or less of the ingested amount is excreted or, conversely, 95% is absorbed. The physical status of the lipid material is said to be of diagnostic importance; for example, pancreatogenous steatorrhea implies excess of neutral fat resulting from a specific lack of this source of lipase. The reliability of the neutral fat-fatty acid ratio is questionable because of other known sources of lipase from the stomach, small intestine, and colon bacteria. The value of quantitation of fecal fat lies not so much in the determination of a specific defect but more in the definitive information that an abnormality exists. The fat content of the feces may be high without alteration in color, odor, consistency, or frequency of bowel movements.

C. Fecal Nitrogen Content.—The normal person given a calculated diet eliminates approximately 8% of ingested nitrogen.[106] In pancreatic insufficiency as much as 50% of the oral intake has been recovered in the stool. This alteration is attributed to specific lack of pancreatic proteolytic enzymes, as nitrogen absorption is thought to be unimpaired in sprue and sprue-like syndromes. Comfort and his co-workers[119] have contested this viewpoint on the basis of carefully conducted balance studies in 11 patients with nontropical sprue. Fecal nitrogen loss as high as 50% of calculated intake was noted. This evidence indicates that fecal nitrogen alterations, at best, indicate nonspecific impaired absorption.

D. Fecal Trypsin Activity.—The principle of this procedure is the demonstration of proteolytic activity of fecal suspensions on x-ray film. A significant response is obtained only with almost complete absence of enzymes. Johnstone[120] has eliminated some

source of error by special dilutions of stool preparations, but contaminating bacterial gelatinases may lead to misinformation. The addition of soybean trypsin inhibitor to a portion of the test solution inactivates pancreatic proteolytic substance, allowing evaluation by control of bacterial gelatinase. This estimation has had extensive trial only in cases of cystic fibrosis of the pancreas.

E. I^{131}-Labeled Protein.—Chinn and co-workers [121] administered test meals containing I^{131}-labeled protein to normal persons and to patients with known pancreatic insufficiency. The theoretical principle was based on the occurrence of normal protein digestion, largely due to pancreatic enzymes, liberating the labeled material for absorption and subsequent urinary excretion. Impaired proteolytic activity, on the other hand, would not allow absorption of the undigested labeled protein, and increased radioactivity would be detected in the feces. Estimations were performed on 72-hour urine and fecal collections. In the 11 normal persons, 61% to 90% of the ingested I^{131} was present in the urine and only 0.5% to 4.8%, in the stool. Five patients with pancreatic insufficiency excreted 20.5% to 55.8% of the radioactive material in the urine, and 24.4% to 63.8% was detected in the feces. These data, particularly the fecal determination, appeared significant in this small series; however, subsequent investigation suggests lack of specificity for pancreatic secretion. The simultaneous administration of pancreatin or other enzyme substitutes may serve to differentiate the increased fecal excretion as to primary enzymatic or absorptive defect.

Detection of Alteration in Digestive Capacity

A. Vitamin A Tolerance.—Several investigators have studied vitamin A absorption in pancreatogenous and other steatorrhea-producing disorders.[122-124] The vitamin A is administered in conjunction with neutral fat. In normal persons, blood vitamin A, fatty acids and chylomicron content reflect the ingested amount. In patients with pancreatic deficiency, only vitamin A, which is not directly dependent on lipolytic enzyme for subsequent absorption, attains control levels. In sprue and sprue-like syndromes, vitamin A, fatty acid, and chylomicron curves [125] remain flat. This procedure will indicate pathologic alteration, but intestinal motility and extrapancreatic sources of lipase introduce errors in specificity.

B. Starch Tolerance.—Althausen and Uyeyama [91] devised a procedure designed to appraise the amylolytic activity of the pancreas. Blood glucose-tolerance curves are compared after separate administration of 100 gm. of starch and 100 gm. of dextrose. Pancreatic amylase deficiency is reflected by curves remaining at approximately the fasting level after starch ingestion, while dextrose produces normal or diabetic-like patterns. If malabsorption is the basic defect, both materials quite frequently fail to evoke alteration in the fasting values. These authors noted comparatively flat post-starch curves in 87% of 23 patients with known pancreatic neoplasm or insufficiency and regard the determination superior to the gelatin tolerance test proposed by West and associates.[126] Our own limited experience and that of others [127] has been less encouraging.

As noted by Fourman [128] and recently reemphasized by Benson and co-workers,[129] an additional tolerance test utilizing a carbohydrate, D-xylose, is of value in the diagnostic evaluation of malabsorption syndromes. This procedure does not estimate digestive capacity but directly parallels small-intestinal absorption, completely independent of enzyme activity. The urinary excretion in the five hours after an oral test dose is measured and correlated with absorption. Urinary xylose levels are uninfluenced by pancreatic defects, but definitely low levels are noted in the other steatorrhea-producing disorders. Pancreatic exocrine insufficiency, then, can be segregated on its failure to impair D-xylose tolerance.

C. I^{131}-Labeled-Fat Meals.—Oral administration of I^{131}-labeled fat and measurement of blood radioactivity at subsequent appropriate intervals has been advocated as a simple and reliable test of pancreatic function.[130] Recently, Shingleton and his associates have reported their results in a series of 30 patients with positive diagnostic confirmation.[131] In the fasting state following thyroid blockade with strong iodine (Lugol's) solution, a calculated emulsified mixture of I^{131}-labeled glycerin trioleate, peanut oil, polysorbate 80 (Tween 80) and barium sulfate was ingested. At intervals of four, five, and six hours, venous blood samples of 2.0 cc. were assayed for radioactivity. Assuming total blood volume to be 7.2% of body weight, the total I^{131} blood content was calculated from the 2.0 cc. aliquots and expressed as percentage of ingested material. Stool collections for 48 hours after test meals were measured for content of labeled material. Of 18 patients with carcinoma of the pancreas and 12 with chronic pancreatitis, only two I^{131} blood curves in each group were in the control range. Of 10 patients with carcinoma and 8 with chronic pancreatitis in whom fecal radioactivity was measured, only one of each group failed to indicate impaired absorption. The authors were of the opinion that the approximately 90% positivity indicated the value of the procedure in pancreatic disease; however, it was noted that minimal alteration of function might pass undetected.

Other investigators have independently confirmed the applicability of radioactive-labeled-fat tolerance tests in various malabsorption states. Beres and co-workers [118] established that the majority of the blood radioactivity eminated was present as inorganic I^{131}; the remainder was lipid-bound. Although it was felt that the iodine-fatty acid bond survived intestinal digestion and absorption completely (the inorganic form is liberated by metabolic utilization [132]), measurement of the lipid-bound fraction of the blood would obviate any such error. McKenna and his colleagues [133] encountered some difficulty obtaining reproducible blood curves in their study and suggested that fecal I^{131} measurements were preferable. Ruffin [134] expressed the opinion that these inconsistent blood levels resulted from variation of gastric emptying, influenced by intake of food immediately prior to the test. As emphasized by all investigators, adequate thyroid blockade by strong iodine solution or syrup of hydriotic acid must be accomplished before iodine-labeled material is administered. Some inconsistency of blood curves may result from variation in renal I^{131} clearance and inconstant or inaccurately estimated radioactive iodine distribution spaces [135]; however, the consensus indicates that still another relatively simple method of appraisal of malabsorption syndromes is available.

D. Estimation of Phospholipid Synthesis with Radioactive Phosphorus.—This determination is still in the preliminary phase; however, the potential value and, more particularly, the alleged specificity for pancreatic disease warrants scrutiny. Nardi [136] has estimated phospholipid synthesis in human subjects through incorporated radioactive phosphorus. The investigation was stimulated by experimental work by Barker and his associates,[137] who found impaired phospholipid formation in pancreatectomized dogs. Blood samples were drawn at intervals for 72 hours subsequent to injection of 300μc. of P^{32} in the form of $NaH_2P^{32}O_4$. Phospholipids were separated from plasma inorganic phosphorus by trichloracetic acid precipitation, and the radioactivity in each fraction was determined. The patients tested included two cases of carcinoma of the pancreas, one of post-total pancreatectomy, two of chronic pancreatitis, only one of whom displayed insufficiency, and two patients without pancreatic disease. There was markedly impaired phospholipid synthesis in patients after pancreatectomy, in patients with pancreatic insufficiency, and in both cases of carcinoma. The normals and the person with chronic pancreatitis

without insufficiency disclosed no abnormality.

The implication that pancreatic disease alters phospholipid synthesis becomes quite involved. The process would commence with ingested lipotrophic factors, particularly methionine and choline, being inhibited from reaching the liver. This deficiency, then, would impair phospholipid formation as reflected by newly formed plasma phospholipid levels. This simple statement of alleged sequence contains an overwhelming number of variables and unknowns. Much more detailed investigation and application will be required before this procedure can be accepted as a test of pancreatic function.

Detection of Abnormalities of Pancreatic Secretion via Duodenal Intubation

Aspiration of duodenal contents and qualitative and quantitative analysis after pancreatic stimulation was reported of diagnostic significance by Agren and his associates,[138] in 1936. These investigators found that secretin evoked the most consistent response. Although standardization and purification of the hormone afforded difficulty initially, this has been accomplished subsequently. Other methods of stimulation, including bethanechol,[139] methacholine,[140] and insulin hypoglycemia,[141,142] have been studied; however, secretin is considered the most effective and reliable preparation.[142-147]

Several sources of error may be introduced by variation in technique. The double-lumen collecting device is passed through the mouth (only in rare instances is local anesthesia necessary) and fluoroscopically guided to the collecting position. It is imperative that complete and separate gastric and duodenal aspiration is obtained. It is only through fluoroscopic control that the necessary placement of the proximal tube in the gastric antrum and the distal tube beyond the papilla of Vater is accomplished. This must be verified prior to and after the test period. Constant suction apparatus is applied to both outlets. Uncontaminated duodenal contents appear clear and bile-stained, having a pH of about 6.0. When these characteristics are identified, a control period of 10 to 20 minutes is observed. Secretin (1.0 clinical unit per kilogram of body weight) is injected intravenously, and 10 to 20 minute collections are taken. Dreiling[147] originally employed an 80-minute survey, but he has stated subsequently that good evidence exists that shorter periods may be adequate. The separation of specimens into 10- and 20-minute aliquots affords some assurance of complete collection, as wide inconsistent variations in volume could indicate inadequate aspiration. The tube is cleared by careful injection of 25 to 50 cc. of air after each designated time period. Each specimen of pancreatic juice is submitted to the following determinations: (1) volume, (2) bicarbonate concentration, (3) enzyme concentration (amylase and lipase), (4) icteric index, (5) guaiac reaction, and (6) cytological studies. Dreiling's results in 123 patients without pancreatic disease are given in Table 2.[148] As hypersecretion is without known significance, only the lower limits of statistical data are included.

TABLE 2.—*Results of Determinations in 123 Patients Without Pancreatic Disease* *

Collection Time, Min.	Total Volume, Cc./Kg.	Maximum HCO_3 Conc., mEq./L.†	Total Amylase, Units/Kg.
30	1.1	91	
60	1.7	91	2.9
80	2.1	91	8.3

* From Dreiling.[148]
† In any 1 specimen.

A. Volume of Secretion.—As the primary effect of secretin is to stimulate volume and bicarbonate secretion, these two estimations are most valuable. The critical volumes of 1.1 cc. per kilogram in 30 minutes or 2.1 cc. per kilogram in 80 minutes will be exceeded unless blockage of pancreatic ducts is present. The extent of diminished volume should be proportionate to the site and degree of obstruction. Of 61 patients with proven pancreatic carcinoma, 53 exhibited decreased volume response in Dreiling's studies.[149] The failures were primarily in

cases with tumors involving the tail of the gland. The volume of secretion is usually low or low normal in chronic relapsing pancreatitis. Of 41 of such patients tested, 30 were shown to have abnormally low volumes.[150] No predictable alteration was noted in acute pancreatitis. Marked dehydration and recently administered medication (in addition to opiates), such as chlorpromazine[151,152] and acetazolamide (Diamox)[153] may result in "false" low volumes.

B. Bicarbonate Concentration.—The maximum bicarbonate concentration should exceed 91 mEq per liter in at least one of the collected samples. In 71 patients with chronic relapsing pancreatitis only 2 exhibited a maximum concentration which exceeded the statistical lower limit, but 28 of the group of 61 carcinoma patients produced normal values. The diagnostic implication of this determination in chronic relapsing pancreatitis should be appreciated.

C. Enzyme Concentration.—The previously accepted hypothesis that enzyme elaboration from the acinar cells occurs in proportionate quantities has been questioned recently.[154] Differential secretion of amylase, lipase, and proteolytic substances on the basis of pancreatic disease has not been demonstrated as yet. This will require further study. Dreiling has assayed only amlyase concentration for statistical normals. As the specific stimulus, secretin, has no primary effect on acinar cells, enzyme has reflected a "washing-out" phenomenon and is said to parallel the secretory volume. Subsequent investigation with an ideal secretagogue (e. g., secretin-pancreozymin) may clarify these unknown features.

D. Concentration of Biliary Pigment.—Several studies have emphasized the importance of the biliary pigment response curve during secretin-pancreatic testing as a reflection of the integrity of the biliary tract.[155-157] Administration of secretin, in the presence of a normal gallbladder and common bile duct, results in visibly detectable diminution of biliary pigment in the duodenal aspirant. This initial low to absent concentration gradually returns to control levels in the ensuing specimens. This normal response is attributed to the reservoir function of the gallbladder and routinely does not occur in patients with a diseased or surgically removed organ. In the latter situations the icteric index of the duodenal fluid remains at control levels or may actually increase. In complete biliary-tract obstruction no pigment is detectable at any time. In certain postcholecystectomy patients partial biliary-tract obstruction due to stricture or stone may be indicated by demonstrating initial pigment diminution from the "reservoir function" of a dilated common bile duct.

E. Cytology of Duodenal Aspiration.—Improved technique and skill in the collection and identification of exfoliated cells obtained by the duodenal aspiration of secretin-induced secretion is becoming of the utmost diagnostic importance. These cytologic techniques, coupled with volume and bicarbonate determinations, are affording 85% accuracy in the diagnosis of pancreatic disease.[157]

Summary and Conclusion

The clinically proven and promising experimental procedures employed in the assessment of pancreatic disease are reviewed. The rather striking reserve function of this digestive organ renders appraisal by any one method hazardous and unreliable. Newer techniques have eliminated some previously laborious estimations; increasing accuracy is being gained through more frequent application. Many incorrect diagnoses result from unfamiliarity with available methodology. Understanding of the important advances in research combined with clinical experience is essential to the more accurate diagnosis and to the subsequent management of pancreatic disease.

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Evolution of Modern Medicine

This section, which has as its primary purpose a review of the contributions of medical investigators and observers of the past to the present-day concepts of disease, is under the special editorship of Dr. Arthur Bloomfield, 2398 Sacramento St., San Francisco 15.

His editorial in the August, 1957, issue of the A. M. A. Archives of Internal Medicine, *gives further detail of what it is hoped to accomplish in these articles. Contributions or comments for this section may be sent directly to Dr. Bloomfield, or to the Chief Editor for transmittal to him.*

Stokes-Adams Disease

An Account of Important Historical Discoveries

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This is a historical account of Stokes-Adams disease, with emphasis on the important advances which have increased our knowledge of the disease in an attempt to discover how these advances came about and the influence they had on medical thinking. Important historical information has been included in many texts on aspects of heart disease, especially those of Hirschfelder [1] and of Lewis,[2] and there have been a few papers devoted exclusively to the history of the disease, notably those of Krumbhaar [3] and Wilhem His Jr.[4] While this manuscript was being prepared a new account has appeared with a somewhat different approach than that used in the present paper.[5]

The clinical picture which arrested the attention of early doctors was the association of striking central nervous system symptoms with the finding of an unusually slow pulse. The attacks themselves were described as fainting fits, epilepsy, or pseudoapoplexy, the latter term intending to describe an apoplectic attack followed almost at once by complete recovery. There was a good deal more interest in this clinical picture than is generally appreciated. Stokes' [6] account is the 10th that has been discovered including descriptions of one or more patients, and the disease was described with increasing frequency throughout the rest of that century (Table). In addition to Stokes and Adams, other important medical figures of the time, among them Richard Bright,[15] Bence Jones,[16] and Charcot,[17] wrote on the subject, and interest has extended into other fields. In 1836 the

Early Descriptions of Stokes-Adams Disease

Author	Year	Cases, No.
Gerbezius *	1691	1
Morgagni [7]	1769	2
Spens [8]	1793	1
Adams [9]	1827	1
Burnett [10]	1827	1
Mayo [11] (Case VII)	1837	1
Gibson [12]	1838	1
Holberton [13]	1840	1
Worthington [14]	1840	2
Stokes [6]	1846	7

* Cited by Morgagni.[7]

London Medical Gazette published an abstract of a paper from the *Veterinarian,* by Pope, describing fainting fits in a horse.[18] There is no note indicating why this was published; perhaps it was because the *Gazette* printed items of general scientific interest along with those of medical importance, but in any case it was an ad-

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mirably simple description of a Stokes-Adams attack in a horse: "The heart at times ceased beating, and the horse fell down," and there was an account of the postmortem findings described as dropsy of the cord. Later there was speculation about the nature of Napoleon's slow pulse in relation to the rumor that he had epileptic fits, but the evidence pointed to the fact that his pulse, although slow, was not within the range usually found in Stokes-Adams disease and that he did not suffer from fits.[19]

Most modern writers accept the belief that the patients described by earlier writers did suffer from Stokes-Adams disease and heart block, but some, notably Thomas Lewis,[2] have expressed doubt that this belief is justified, mainly because there were no graphic records to display the cardiac mechanism. This point of view seems short-sighted and deserves further comment, especially because of the occasional use of the term to describe syncopal attacks unrelated to heart block. The clinical descriptions of early writers were often beautifully clear, and there are few diseases in which the same clinical picture might be reproduced. Moreover, there was a high mortality among the early cases, something not apt to occur with syncopal attacks from other causes, and finally Stokes and others noted physical findings which leave no doubt that their patients had heart block. Stokes[6] described what must have been auricular sounds and the typical appearance of the jugular pulse in patients with complete heart block. While his description of the auricular sounds falls somewhat short of being convincing, this is not true of his description of the jugular pulse. In recording the observations on his first patient, he noted, "The cardiac phenomena remain as before but a new symptom has appeared, namely, a very remarkable pulsation in the right jugular vein. This is most evident when the patient is lying down. The number of reflex pulsations is difficult to be established, but they are more than double the number of the manifest ventricular contractions. About every third pulsation is very strong and sudden and may be seen at a distance; the remaining waves are much less distinct . . . ." The smaller waves he observed were without doubt the independent auricular waves often seen in the jugular pulse in patients with heart block; the strong ones, those which occur in complete heart block when auricular and ventricular systole coincide. If one agrees that Stokes' patient had heart block, then it is clear that the term Stokes-Adams disease should be used only to describe syncopal attacks which occur in patients with heart block. It should not be used, as it sometimes is, to describe syncopal attacks occurring with other cardiac conditions, especially those which take place with ventricular arrhythmias. This turns out to have some practical meaning. It has been shown in persons with heart block and syncopal attacks related to ventricular arrhythmias that quinidine or procaineamide may induce attacks,[20,21] while in the same arrhythmia occurring in the absence of heart block quinidine and procaineamide are useful therapeutic agents.

The disease is called Adams-Stokes about as often as Stokes-Adams, and sometimes Morgagni's name is added. Fortunately the meaning of any of these names is clear, for it is difficult to bring forth convincing arguments in support of a particular order or choice of names. "Permanent slow pulse" was used as a name for a time in France and appeared in the "Index-Catalogue of the Library of the Surgeon General's Office" after Charcot[17] used this phrase in the title of a paper. Stokes[6] used the phrase "permanently slow pulse" in the title of his paper, but this was never used as a name for the disease. The first actual proposal for a name came in 1889, from Huchard,[22] proposed by him in the following sentence: "The three part syndrome, first noted by Adams, then by Stokes, from whence comes the name, disease of Adams or of Stokes-Adams which I propose to give it." He gives no reason for using Stokes'

name first, nor is it clear that it was ever called the disease of Adams. The next year, when he again proposed the name Stokes-Adams, he followed this by adding "or the disease of Adams" and explained that his choice of names was shorter and better than the name taken from Charcot because all patients did not have a slow pulse.[23] Huchard would have simplified matters very much if he had been more explicit about his reasons for using Stokes' name first. Perhaps it was because Stokes reported several cases and because of his plea for recognition of the case described by Adams. His plea was not a forceful one. Referring to Adams' case, he writes: "The attention of subsequent writers on diseases of the heart, has not been sufficiently directed to this case which is an example of a very curious, and, there is reason to believe, special combination of symptoms." Presumably those who use Adams' name first do so because his account was much earlier, and it is interesting to note that His Jr.,[24] after carefully noting Huchard's[22] proposal, uses Adams-Stokes in the text and title of his paper. The claims of earlier writers, Gerbezius (cited by Morgagni[7]), Morgagni,[7] Spens,[8] and Burnett[10] for priority could be disposed of by arguing that they did not recognize that the disturbance was a form of heart disease. All followed Morgagni in believing it to be a form of epilepsy. There would still be no effective way of dealing with the names of those who describe the disease between the time Adams and Stokes, especially Gibson[12] and Worthington,[14] who gave excellent accounts of the disease.

With these difficulties in arriving at a just decision about who should be honored, the physician is entitled to his own choice of names. In this account Stokes-Adams is used because it is the first name proposed.

Early writers had few facts on which to base an understanding of the mechanism of the disease. Some of Harvey's[25] work should have given useful clues, but there is no indication that it did. He was aware of the regular sequence of auricular and ventricular contraction and appreciated that this was a well-ordered mechanism. Moreover, he had observed what amounted to heart block in the dying heart of an animal, noting first a pause between auricular and ventricular contraction, later a response of the ventricle after two or three auricular contractions, and finally continuation of auricular contraction after the ventricles had ceased to beat. Morgagni was the first to note that cessation of the pulse was followed by an attack but did not comment about a causal relation between this and the symptoms. Gibson,[12] after observing the same phenomenon, attempted to explain it on the basis of obstruction in the iliac arteries and dilatation of the aorta found in the autopsy of his patient. The heart, unable to force more blood into the arteries, stopped. A case of Holberton's[13] was of especial interest because it was often quoted in support of the view that the disease originated in the nervous system and because it provoked the first discussion among physicians about the cause of the disease. His patient, a man who sustained a severe neck injury in a fall from a horse two years before the onset of fits and slow pulse, was found at autopsy to have narrowing of the anteroposterior diameter of the foramen magnum and compression of the medulla. He attributed the symptoms to the lesion in the nervous system. Some discussion was published with Holberton's paper, but of special interest were some comments made later by Worthington.[14,26] In his first paper Worthington reported two cases of his own and was critical of Holberton's conclusions because he had not paid enough attention to the heart. In the second paper he reported the autopsy findings on the second of his cases and made a shrewd observation as to the cause of the disease. Autopsy showed the coronary arteries to be more or less completely obstructed and "turned to bone." Referring to this case and to Holberton's views, Worthington remarked, "I am still inclined

to the opinion that it [the slow pulse] usually occurs independent of any disease about the medulla; and that insufficient and perverted nutrition of the substance of the heart may be regarded as an and by no means uncommon cause originating in ossification of the coronary arteries." Stokes [6] had little to say about the mechanism. He thought that the semibeats, a term he used to describe what were probably auricular sounds, were some sort of feeble ventricular contraction. Stokes refers to cases described to him by colleagues but does not refer to any of the reports published between Adams' report and his own, a fact probably related to the state of medical libraries at the time rather than to indifference on the part of the author to the work of others.

After Stokes and before substantial experimental contributions, Ogle [27,28] and Charcot [17] made noteworthy observations. Ogle, in two papers published in 1857 and 1864, called attention to a similarity in the pathological findings in his case and in those of others, noting fibrinous deposits in the heart walls. Although he leaned toward the view that this lesion was important because it interferred with the sympathetic ganglia of the heart, his most noteworthy contribution was the first clear statement of the problem. He said in part, ". . . this fact becomes naturally associated in one's mind with those experiments which were performed on the frog and other animals, in which it was demonstrated, that a very clear and sensible connection existed between the integrity of the great transverse fissure of the heart and the movements of the ventricles." After such a good beginning one is disappointed to find the same author in 1897 expressing the view that the disease was due to interference with the intrinsic nerves and ganglia of the heart.[19] Charcot [17] included the disease in his lectures on diseases of the nervous system. Although he did not exclude the heart as the cause, he argued from knowledge about disease and injury to the cervical cord or medulla and from Holberton's case that the cause of the disease was usually in the nervous system. However, he was unable to find evidence of disease of the cervical cord or medulla in his patients.

Although early writers made little progress toward understanding the nature of the disease, they did describe the attack so clearly that little can be added. In addition they noted some special symptoms which deserve attention today. One of Morgagni's [7] patients experienced attacks on turning the head from side to side, another noted palpitation preceding the attack, as did one of Stokes' [6] patients. Spens [8] noted in the patient he described that attacks were apt to come during eating or swallowing, a relationship which may occur in syncopal attacks other than Stokes-Adams disease but also occasionally when there is heart block.[29]

The first important contribution to the physiology and anatomy of the disease were made by Gaskell [30] and by His Jr.[31] Each undertook his studies because of skepticism about the current views of cardiac physiology, specifically the point that cardiac conduction took place by way of the nervous system. Others soon made similar observations; but theirs were the first, and their basic observations have withstood the test of time and disposed of the neurogenic theory of cardiac conduction for good.

Gaskell's [30] work is well known, but perhaps the extent of his observations has been forgotten. In the tortoise, with a carefully planned series of experiments, he excluded the nervous system as the means of auriculoventricular conduction and showed by physiological experiments that conduction took place through auricular muscle and then across the auriculoventricular groove. He noted on histological examination interchange of fibers between auricle and ventricle at the auriculoventricular groove and a difference in structure of the interchanging fibers as compared with other cardiac fibers. He proposed that the difference in structure was related to the function of delay of conduction from auricle to ven-

tricle at the auriculoventricular groove. He also introduced the term block to describe the phenomenon he had observed, a term which he states was borrowed from Romanes, who had used it for a somewhat different purpose.

His Jr.[31] approached the question in an entirely different way. As he explained later, he was led to study the problem as a result of conversations with Krehl and Romberg in which doubts were expressed about the part of the nervous system in cardiac conduction.[4] Because he had been trained in embryology by his father, it occurred to him to observe the development of the nervous system in relation to the time of appearance of the heart beat in the embryo. He was able to show in vertebrate embryos the appearance of the heart beat before the heart received spinal nerves or ganglia. He then searched for and found in animal and human material the connection between the auricles and ventricles which is now known as the bundle of His. For a discussion of the merits of Kent's claim to priority in the matter of the bundle, one should read Erlanger's [32] comments.

After completion of his anatomical studies, His Jr. produced experimental heart block in the rabbit. A brief report of these experiments was published in 1895, but there were no details or illustrations of the tracings demonstrating heart block or sections of the heart.[33] However, in his historical account of the atrioventricular bundle published in 1933 he reported these experiments in some detail and published illustrations of the tracings and sections.[4] Block was produced by inserting a cataract knife into the right auricle of a rabbit, probing for the site of the auriculoventricular bundle and making an incision when he thought he had located the correct area. In 18 experiments he produced block 3 times, in 1 only temporarily, but the tracings of the successful experiments, reproduced in Figure 1, and of the sections, reproduced in Figure 2, show that he had induced block and incised the auriculoventricular bundle. An illustration showing the sites of the successful and unsuccessful incisions was also published. It is curious that he waited so long before publishing his evidence, and he gives no reason for this.

In 1899, His Jr.[24] published an account of the disease based on an extensive clinical observation of a patient, including many tracings, among them the first to illustrate the cardiac mechanism during a Stokes-Adams attack. This was also the first paper in which the terms Adams-Stokes disease and heart block appear together in the title. Jugular pulse tracings between attacks, reproduced in Figure 3, show complete block, and the tracings during the attack, reproduced in Figure 4, while lacking in artistic quality and a record of time intervals, show without doubt a long period of auricular activity while the radial pulse was absent. His Jr. made two other interesting observations on his patient. The first was a humming murmur at the base of the heart, synchronous with the auricular pulsations in the neck. This still seems to be a unique observation; it is unlikely that this is the auricular diastolic murmur described by Rytand,[34] since these murmurs are not high-pitched and do not occur at the base. He also recorded a Stokes-Adams attack during Cheyne-Stokes respiration, noting that it began with the period of hyperpnea. With this paper His Jr. brought almost to completion a circle of original studies of heart block; he was the first to discover the pathway for conduction, the first to produce experimental block in the mammalian heart, and the first to obtain graphic records of the mechanism during a Stokes-Adams attack. He was frustrated in his attempts to obtain a postmortem examination. The patient died at home, and an autopsy could not be obtained.

Two other events in this era deserve notice. In 1875, Galabin [35] in a study of the cardiogram in mitral stenosis, included tracings reproduced in Figure 5, showing complete heart block. The patient was not thought to have mitral stenosis, but Galabin

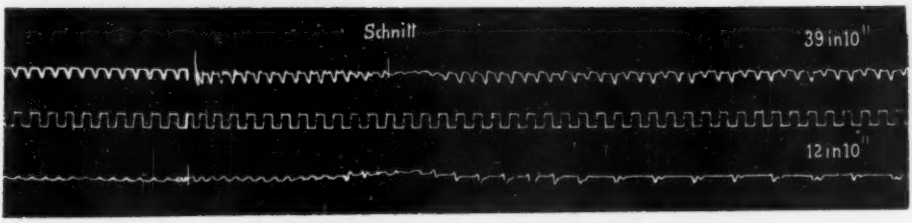

Fig. 1.—Experimental production of heart block in the rabbit. Upper tracing, auricle. Middle tracing, time. Lower tracing, ventricle. Complete heart block occurs when the bundle is cut. Reproduced with permission from His, W.: *Klin. Wchnschr.* 12:569, 1933.

Fig. 2.—Longitudinal section through the auriculoventricular bundle from the experiment illustrated in Figure 1. *A*, auricle. *B*, ventricular septum. Arrow points to incision in the bundle. Reproduced with permission from His, W.: *Klin. Wchnschr.* 12:569, 1933.

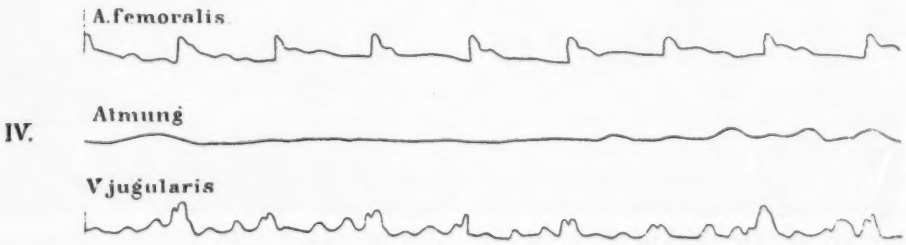

Fig. 3.—Femoral and jugular pulse tracings from His' patient with Stokes-Adams disease and heart block. Reproduced with permission from His, W., Jr.: *Deutsches Arch. klin. Med.* 64:316, 1899.

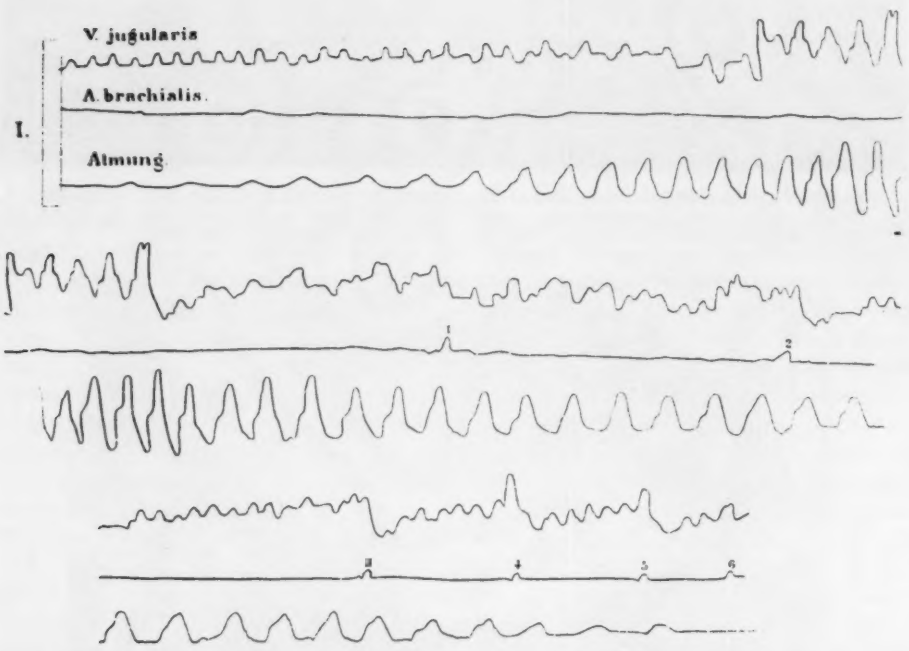

Fig. 4.—Record during Stokes-Adams attack from the patient whose tracings during heart block are reproduced in Figure 3. The original continuous tracings have been divided into three sections, each with brachial, respiratory, and jugular tracings. The record begins during the attack. In the upper section the jugular pulse shows "a" waves but no "c" waves, and there is no brachial pulse. In the middle section the jugular tracing is disturbed by movement of the patient, and toward the end there are two brachial pulses. In the lower section "a" waves are again seen, and beginning in the central portion brachial pulses return. Reproduced with permission from His, W., Jr.: *Deutsches Arch. klin. Med.* 64:316, 1899.

published the tracings because of their unusual nature. He knew that the "a" waves represented auricular activity but offered no other analysis of the tracing. In 1885, Chauveau [36] published cardiograms and jugular pulse tracings from a patient with an unusually slow pulse. These tracings, interpreted by the author as showing dissociation of the auricular and ventricular rhythms, were the first tracings of heart block to be identified by the author as such. The cardiograms are reproduced in Figure 6. Chauveau turned to the laboratory to explain what he had observed and using the horse as the experimental animal showed that stimulation of the peripheral end of the vagus would induce block and concluded that this was the cause of the dissociation he had observed in his patient.

With the publication of His Jr.'s [24] clinical report in 1899, the stage would appear to have been set for the recognition and appreciation by clinicians of the mechanisms responsible for the disease and especially the relationship between heart block and Stokes-Adams disease. Confirmation of all aspects of his work was needed, but as one looks at the evidence now it appears that there were at least grounds for lively speculation about the validity of his conclusions. However, appreciation of the nature of the disease did not take place for several years, and when it did it is difficult to discover how it came about. Midway during this period Osler [37] published a clinical account of 12 cases of Stokes-Adams disease, including beautiful descriptions of physical signs during an attack in one pa-

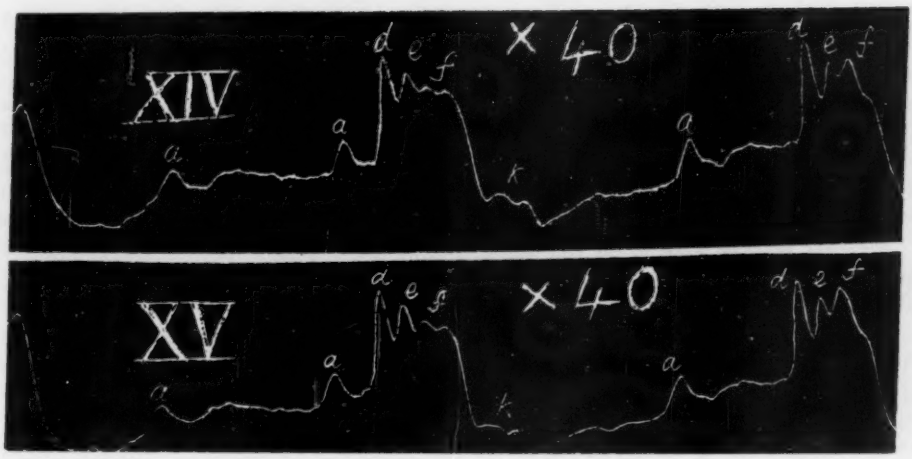

Fig. 5.—Cardiogram showing complete heart block. In the text the "a" waves were identified as being due to auricular activity. Reproduced with permission from Galabin, A. L.: *Guy's Hosp. Rep.* 20:261, 1875.

tient and a long discussion about possible causes of the disease. Coming from Osler it is probably a good representation of medical thinking at the time. In Case 5 of his series small regular systolic impulses were seen in the fourth and fifth interspaces at exactly 100 to the minute, along with faint systolic sounds at the same rate. During two attacks which lasted about one-half minute these impulses were observed to continue throughout the attack. The attack itself seems to have been due to marked

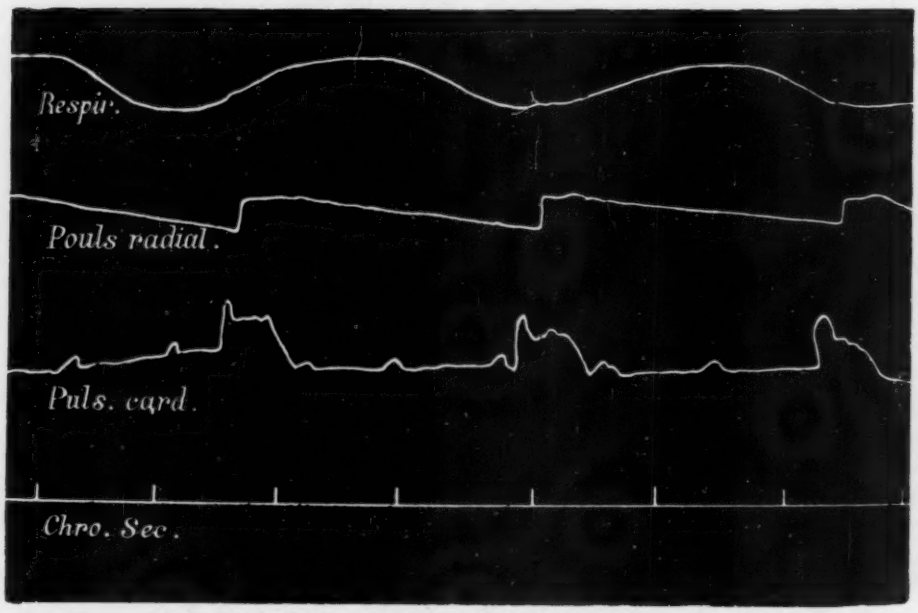

Fig. 6.—Cardiogram showing complete heart block. The small waves in the cardiac pulse were identified as being due to auricular activity. Reproduced from Chauveau, A.: *Rev. méd., Paris* 5:161, 1885.

slowing of the pulse—the heart rate was 12—rather than to a long period of ventricular standstill. On a number of other occasions in the absence of a Stokes-Adams attack the same small regular impulses were observed and counted. Jugular waves at a rapid rate were also seen but not clearly enough to be carefully observed.

It is not possible here to do full justice to Osler's long discussion. He was familiar with the work of Chauveau, His Jr., Gaskell, and others and clearly understood the concept of heart block, but if there is a single characteristic to the discussion it is caution, best shown by quoting a sentence from the concluding paragraph. "Is the essential factor centered in the medulla or in the ganglia of the heart or in the automatic mechanism of the muscle itself, or in the auriculoventricular Bundle of His, Jr., or in the Kronecker coordination center? I do not think we know." A good deal of his discussion is concerned with the findings in the patient just described, in particular about the small impulses seen on the chest wall. Since it seems certain that what he had noted on physical examination in this patient was precisely what Chauveau [36] had recorded and described in his case, it is curious that Osler does not at least consider that he was observing the same phenomenon, especially during the attack. The subtitle to Case 5, "Remarkable Discrepancy Between Heart and Pulse," and several references in the text to feeble or abortive ventricular contractions suggest that if he was inclined to any view about the nature of the impulses he observed it was that they represented some sort of ventricular contraction. Osler mentions that he had attempted unsuccessfully to record the jugular pulse in this patient, and it is clear that he was not satisfied with the situation as he understood it, for he was later to give Erlanger [38] the opportunity to make important observations in another patient.

During the same era others confirmed His Jr.'s [31] discovery of the auriculoventricular bundle [39,40] and succeeded in producing heart block in animals by a section of the bundle.[41,42] Hay,[43] in 1905, in describing a case of heart block with Stokes-Adams disease published jugular pulse tracings during heart block and mentioned a relationship between heart block and Stokes-Adams disease but gave no reason for doing so. In 1906 what amounted to a symposium on heart block was held at the annual meeting of the British Medical Association. There was nearly general agreement about heart block. Aschoff [44] spoke about the conduction system and commented about two cases with heart block in which a lesion of the bundle was found. Mackenzie [45] presented a case with jugular pulse tracings, and Erlanger [46] discussed the physiology of heart block. Only Erlanger and Aschoff, the only participants who had studied fundamental aspects of the disease, pointed out the relationship between heart block and Stokes-Adams disease. Sir James Barr,[47] in describing a case of Stokes-Adams disease, disagreed with nearly everyone. In his comments he stated: "The so-called myogenic stimulus or the production of the stimulus of the contraction as a function of the cardiac muscle is, so far as I know, purely an inference, possibly a figment of the imagination which exists in the brain of certain writers rather than in the cardiac muscles." His comments must have provoked some discussion, but unfortunately none was published.

Erlanger, who contributed more than any other person toward putting knowledge about the disease on a sound basis, began his work in the same period. Others had only limited success in interrupting the bundle of His in animals, but Erlanger [48,49] developed a method which nearly always succeeded. With this he was able to make many observations during acute experiments in dogs and later to produce chronic heart block in the dog.[50] During the course of this work he was able to observe a case of heart block in man, the case referred to above in which Osler, knowing that Erlanger had devised apparatus for recording

blood pressure in man, interested him in making the observations.[51] In man and in dogs the cardiac mechanism was observed by means of jugular pulse tracings and cardiograms. In his patient he made an amazing number of observations, including varying degrees of blocks as well as complete blocks, Stokes-Adams attacks, the effect of extrinsic factors such as exercise and atropine, and, of special interest even today, an increase in auricular rate immediately preceding ventricular standstill and the induction of standstill by breath holding. Many important observations were made in his experiments on the dog. Of special interest were his observations on the lengthening of the interauricular period following ventricular systole and the observation in chronic heart block in the dog of what was without doubt ventricular tachycardia followed by a ventricular standstill. In three animals what was described as group beating of the ventricles occurred: in two, for only a few days after operation, but in the third animal, 40 days after induction of block. The group beating consisted of a rapid slightly irregular ventricular rhythm with a rate of from 160 to 210, ending with ventricular standstill. During one such episode cardiograms obtained during the rapid ventricular rhythm showed independent auricular rhythm at the usual auricular rate. In this animal attacks of ventricular rhythm could be induced by exercise, and the symptoms of the Stokes-Adams attack coincided with the period of ventricular standstill which followed cessation of the rapid ventricular rhythm. Thus the pattern was the same as one of those described later in man by Parkinson et al.,[52] ventricular tachycardia followed by ventricular standstill.

By 1908 there appeared to be general agreement about the nature of the disease. In that year Lewis,[53] after scrutinizing most of the published information, stated, "It has been recognized for over 80 years but it is only quite recently that evidence has so far accumulated as to point to the condition as being due in certain instances to the pathological lesion of the auriculoventricular bundle." The same year, Krumbhaar,[3] in his historical account, remarked that most of the knowledge about the disease had been gained since the graduating class had entered medical school.

This agreement seems to have developed more through the impact of repeated studies rather than through the influence of a single investigator. In retrospect it seems that clinicians, at least, were slow to appreciate the importance of the fundamental studies that had been done, and one gets the impression that Erlanger's work did not receive the attention that it deserved. This seems especially true insofar as clinicians were concerned about his paper on heart block in man.[38] Perhaps this was because it was published in a journal of limited circulation and because of the arrangement of the title. The main title referred to the physiology of heart block in mammals; only the subtitle indicated that one of the papers of this series was about heart block in man. The situation reminds one of the spread of knowledge about coronary thrombosis and myocardial infarction. Here the situation was much simpler, involving only correlation of gross pathological findings with a striking clinical picture, but as Levine[54] has stated in commenting about Herrick's[55] publication: "Curiously enough (it) did not produce the desired effect for it aroused no interest." It was not until further reports by Herrick and rediscovery of the disease by others that physicians became aware of the clinical picture. The reasons for this are obscure. They seem related to what Zeman[56] has recently written about as "The Nature of Medical Error."

With the completion of Erlanger's work most of the important discoveries of historical interest had been made. When physicians finally became aware of heart block and its relation to Stokes-Adams disease the terms were used synonymously, but in 1909 Volhard[57] pointed out that all patients

with heart block did not have Stokes-Adams disease. With the introduction of the electrocardiograph much new information was discovered about the details of the cardiac mechanism, especially the mechanism during the attack. Kerr and Bender [58] were probably the first to record ventricular arrhythmia during an attack in man. In 1941, Parkinson, Papp, and Evans [52] collected from their own material and from the literature 64 cases in which an electrocardiogram had been made during a Stokes-Adams attack. In approximately one-half of the cases the attack was due to ventricular tachycardia or fibrillation, either alone or followed by ventricular standstill. In the remaining cases it was due to ventricular standstill or to extreme slowing of the ventricle.

Concluding Remarks

Development of knowledge about Stokes-Adams disease took place over almost a century, beginning with clinical interest in a striking syndrome, often described with beautiful clarity despite lack of knowledge on the part of the observers about the nature of the disease. Explanation of the basic causes of the disease came about first through the efforts of three men—Gaskell, His Jr., and Erlanger—all of whom undertook their work because of skepticism about previous beliefs regarding cardiac physiology and anatomy or because of their curiosity about fundamental aspects of the cardiac mechanism. Appreciation of their work was slow in coming, especially by clinicians, for reasons that are not entirely clear, a situation that reminds one that it is possible at any time to overlook important fundamental observations. Finally, it should be emphasized that there are good historical reasons for limiting the use of the term Stokes-Adams disease to describe syncopal attacks which occur in heart block.

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Contributions of Basic Science to Modern Medicine

Vitamin B_6 in Internal Medicine

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Introduction

Vitamin B_6 is required as a growth factor by a wide variety of bacteria, yeasts, and molds and for the nutrition of all species of animals studied. In man it is an essential nutrient, and deficiency of vitamin B_6 results in characteristic alterations of the skin, mucous membranes, and nervous system. Like other vitamins, vitamin B_6 functions as a coenzyme of importance in a wide variety of enzyme systems concerned with intermediary metabolism.

Vitamin B_6 is now used as a class name to include all compounds having vitamin B_6 activity. These include pyridoxine, pyridoxal, and pyridoxamine. To Gyorgy [1] (1934) goes the credit for establishing vitamin B_6 as an entity in the vitamin B complex. Within two years of the original observations of Gyorgy, five independent reports announced the isolation of pyridoxine from various natural materials.[2-6] In 1939, Harris and Folkers [7] successfully synthesized pyridoxine. Snell [8] recognized the existence of other forms of pyridoxine (Fig. 1). Harris,[9,10] using Snell's suggestion of an amine or aldehyde derivative of pyridoxine, successfully synthesized pyridoxamine and pyridoxal. Oxidation of the 4-hydroxymethyl group of pyridoxine to an aldehyde yields pyridoxal. Replacement of the hydroxy group by an amine group results in the formation of pyridoxamine. These three forms of vitamin B_6, as well as at least two conjugated or bound forms, pyridoxal phosphate and pyridoxamine phosphate, occur in biological products.

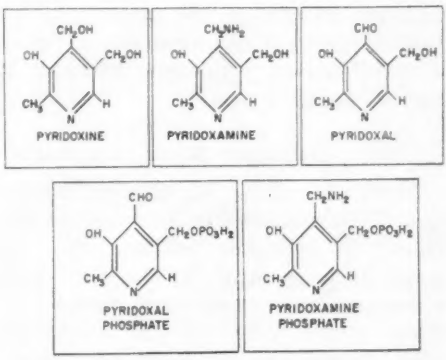

Fig. 1.—The structure of naturally occurring vitamin B_6 derivatives.

Vitamin B_6 is concerned with the activity of a wide variety of enzyme systems. The coenzyme is the 5-phosphate of pyridoxal. The various members of the vitamin B_6 group—pyridoxine, pyridoxal, pyridoxamine—owe their vitamin activity to the ability of the organism to convert them into the enzymatically active form, pyridoxal-5-phosphate. The chemical reactions catalyzed by vitamin B_6 have little in common, except that for the most part they involve action of one sort or another upon amino acids. Thus pyridoxal-5-phosphate serves as a coenzyme for amino acid decarboxylases, transminases, desulfurases, and racimases in many forms of bacteria and in

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animals including man.[11,12] Vitamin B_6 is also essential for the metabolic conversion of tryptophan to quinoline derivatives and nicotinic acid.[11,12] When there is a deficiency of vitamin B_6, intermediary steps of tryptophan metabolism are altered, the conversion of tryptophan to the pyridine coenzymes and N^1-methylnicotinamide is restricted, and intermediary metabolites of tryptophan, including xanthurenic acid, are excreted in the urine.[13,14] This latter biochemical aberration has served as a useful test of pyridoxine deficiency in animals including man.[15]

Vitamin-Deficiency States in Animals

In the rat, vitamin B_6 has a relationship also to fat and essential fatty acid metabolism. In pyridoxine-deficient rats, total body fat is decreased.[16] The fatty acids present are more unsaturated, with a relatively greater percentage of arachidonic acid. Witten and Holman [17] have shown that vitamin B_6 is necessary for the conversion of linoleic acid to arachidonic acid in rats. In addition, the administration of polyunsaturated fatty acids, linoleic or arachidonic acids, is effective in the treatment of the fat depletion and dermatitis in pyridoxine-deficient rats.

Gastrointestinal tract organisms manufacture a significant amount of vitamin B_6. In rats this supply of the vitamin appears to be absorbed and utilized.[18,19] The administration of sulfonamides in conjunction with a pyridoxine-deficient diet significantly reduces the weight gain of rats, while the addition of pyridoxine to the sulfonamide-containing diet restores the growth rate.[19-21] Alteration of the type of carbohydrate in the diet of both rats and chicks has been demonstrated to stimulate bacterial synthesis of pyridoxine. Subsequent absorption of this vitamin from the intestine is associated with improvement in growth and increased urinary excretion of 4-pyridoxic acid.

It is simple to induce vitamin B_6-deficiency symptoms in animals by means of deficient synthetic diets and/or pyridoxine antagonists. The manifestations of deficiency vary with the species of animal, but a few general symptoms seem to be common to most animals. In general, vitamin B_6 deficiency retards growth and induces anemia, epileptiform fits, and scaly reddened lesions of the skin.

The rat has been extensively studied. Poor weight gain is one of the earliest manifestations of vitamin B_6 deficiency. A symmetrical dermatitis or acrodynia occurs, involving the tail, paws, nose, mouth, and ears. Animals given deficient diets for long periods of time develop hyperexcitability, circular running, and convulsive seizures followed by coma. Microcytic anemia may occur occasionally. There is a striking reduction in the ability of the animal to form antibodies when potential antigens are injected.[22-26]

Of particular interest are the observations of Rinehart and Greenberg [27] on the effects of dietary vitamin B_6 deficiency on the circulatory system of the rhesus monkey. Sclerotic lesions developed in the arteries of all deficient animals and were prominent in the coronary arteries as well as in those of the kidneys and pancreas. The experimental lesions bore a close similarity to arteriosclerosis as it occurs spontaneously in man. Fatty metamorphosis and cirrhosis of the liver were not uncommon in these animals.

Anemia is a manifestation of vitamin B_6 deficiency in all mammalian species adequately studied. In swine a microcytic hypochromic anemia occurs regularly.[28] It is associated with high concentrations of plasma iron and hemosiderosis of the liver, spleen, and bone marrow. There is decreased utilization of these iron stores for the formation of hemoglobin. All of these abnormalities are corrected by the administration of pyridoxine.

Absorption

Vitamin B_6 is absorbed rapidly and completely by human beings, though the part of

the intestine through which maximum absorption occurs is not known. Scudi and his associates [29] showed that total urinary excretion of vitamin B_6 following administration of 50 to 100 mg. of pyridoxine was closely similar whether the vitamin was given orally or by intravenous injection. After feeding of each of the three forms of the vitamin (pyridoxine, pyridoxamine, and pyridoxal) to human subjects, the maximum increase in the urinary output of the form fed occurs within two to five hours after ingestion, with a return to the normal value within eight hours.[30] Møller [31] found no significant differences between fecal elimination of vitamin B_6 in experimental animals given a vitamin B_6-free diet and control animals fed vitamin B_6. These results indicate that vitamin B_6, given to the controls, must have been completely absorbed or broken down in the intestinal tract.

Complete balance studies on babies and pigs have suggested that bacterial synthesis of vitamin B_6 takes place in the gastrointestinal tract. When fecal excretion is added to the urinary output of the vitamin, total elimination of vitamin B_6 is significantly larger than the intake. Incubation of the feces of the pigs caused an average rise in the vitamin B_6 content of 63%.[31] The amount of the vitamin found in the feces must therefore have its origin from bacterial synthesis in the digestive tract. Although rats make use of this source of supply,[18] it is doubtful that human beings do. Sarett,[32] using 4 to 6 gm. of oral streptomycin a day, inhibited the growth of gastrointestinal organisms in one human subject studied. But urinary excretion of vitamin B_6 compounds was found to be unaffected. Reports from Italy of results quite opposite to these must be criticized because antibiotics used to destroy intestinal organisms were easily absorbed and excreted in the urine and stool.[33,34] They undoubtedly had a profound inhibitory effect on the micro-organism Streptococcus faecalis, used to measure vitamin B_6.

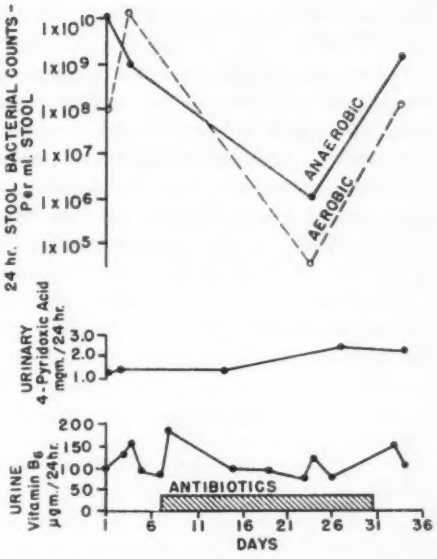

Fig. 2.—Effects of reduction in gastrointestinal organisms of human beings on the excretion of vitamin B_6 metabolites in the urine.

Work from our laboratory also suggests that little, if any, vitamin B_6 is obtained from gastrointestinal organisms by human beings. Two patients from the orthopedic service of the Cincinnati General Hospital were given a 22-day course of poorly absorbed antibiotics administered orally. They were maintained with a regular hospital diet containing between 1 to 2 mg. vitamin B_6 per day without vitamin supplements. One patient (represented in Fig. 2) was admitted with postmenopausal osteoporosis, and the other, a 23-year-old man, had sustained a fracture of his tibia. Twenty-four-hour urinary vitamin B_6 and 4-pyridoxic acid levels were measured together with counts of aerobic and anaerobic bacteria in 24-hour stool collections. Each day polymixin B sulfate, 200 mg.; bacitracin, 20,000 units, and neomycin, 3.4 gm., were used.* A substantial drop in the total bacterial counts of the stools occurred, but no effect was

* Mixtures of these antibiotics were furnished by Reed & Carnrick (neomycin and bacitracin [Neobacin]) and Burroughs Wellcome & Company, Inc. (polymixin B sulfate [Aerosporin Sulfate]).

noted on the urinary excretion of vitamin B_6 metabolites (Fig. 2).

Recent data reveal that adult persons in the U. S. have very low pyridoxal phosphate blood and leukocyte levels compared with those found in infants and in most laboratory animals.[35] Now the question is being raised as to whether the American diet is actually as adequate in vitamin B_6 as we have heretofore thought. If this is true, vitamin B_6 may play a most important role in the medical investigation and practice of the future.

Excretion

Most of the excretion of vitamin B_6 compounds occurs in the urine, where the principal biologically active forms are pyridoxal and pyridoxamine. There is almost no pyridoxine.[30] The total amount of these vitamins in the urine as measured by the microbiological methods using the yeast, Saccharomyces carlsbergiensis, varies from between 0.2 and 0.3 mg/24 hours. Rabinowitz and Snell found that the 4-pyridoxic acid is the chief excretory product. This substance can be measured only by a photofluorometric method.[30] Since this method measures fluorescent substances other than 4-pyridoxic acid, erroneous values may be obtained. Sarett[36] noted that many foods contain substances which fluoresce under conditions used for analysis of 4-pyridoxic acid. After test doses of pyridoxine compounds the interfering substances are eliminated by dilution, thus giving the test validity for comparing the effects of two levels of vitamin B_6 administered or for estimating the results of a pyridoxine load. Sarett found the average urinary 4-pyridoxic acid level in persons on a normal diet to be 3.4 mg. per day. Mueller and Vilter[37] found an average of 3.9 mg/24 hours in eight subjects. But data suggest that these figures are much too high for the reasons noted above. Rabinowitz and Snell,[30] studying urinary excretion of vitamin B_6 metabolites in normal human subjects, found the highest recovery of the four products measured (pyridoxine, pyridoxamine, pyridoxal, and 4-pyridoxic acid) to be 70% when pyridoxal was fed; 45% of pyridoxine and only 31% of the pyridoxamine could be recovered under similar circumstances. These discrepancies in excretion could not be explained.

Several questions in vitamin B_6 metabolism therefore remain to be answered. Are the sums of the known excretory products an accurate expression of the total vitamin B_6 metabolism; does gastrointestinal synthesis play a role; do our analytical methods for vitamin B_6, especially for 4-pyridoxic acid, measure nonspecific substances, or are there still unidentified metabolic products which are formed from vitamin B_6 and excreted? This latter explanation seems most likely.

Internists are interested in animal investigations and studies on human physiology primarily in relationship to their bearing on human disease. Many studies on the clinical manifestations and pathophysiologic results of vitamin B_6-deficiency states have been carried out in human beings. The following sections include summaries of these studies performed in many different laboratories, including our own, together with new data from our laboratory described in greater detail.

Vitamin-Deficiency States in Human Beings

In 1950 Snyderman and associates[38] studied two hydrocephalic infants maintained on a synthetic diet devoid of vitamin B_6. After 76 days, convulsions developed in one of these infants; after 130 days, a microcytic hypochromic anemia developed in the other. Both excreted large amounts of xanthurenic acid in the urine after a load test of tryptophan. All of the abnormalities disappeared after pyridoxine was administered.

Between 1951 and 1954, a considerable number of infants given a vitamin B_6-deficient commercial liquid-milk formula, containing only about 60μg. of vitamin B_6 per liter and with the fatty acid source changed

from cocoanut oil to palm oil at the same time, developed seizures which were rapidly controlled by a change in the formula or supplementation with vitamin B_6.[39-41] Electroencephalographic abnormalities found in these infants were corrected within several minutes by parenteral administration of vitamin B_6.

The relationship between vitamin B_6 and epileptic seizures is not a simple one. That vitamin B_6 metabolism is important for neuronal tissue has been well demonstrated in infants deprived of vitamin B_6. Hunt[42] described an infant with seizures which were dependent for control on continuing doses of vitamin B_6. This patient and his mother were not vitamin B_6-deficient as demonstrated by normal urine xanthurenic acid levels. The mother had been treated with pyridoxine for nausea and vomiting of pregnancy prior to delivery of the child. Studies on vitamin B_6-deficient suckling rats and pigs are consistent with reports on human infants developing convulsions after being deprived of vitamin B_6. Without exception, these animals have responded to supplements of vitamin B_6, but no histological lesions have been found in their brains.[43] In addition, the two vitamin B_6 antagonists, 4-desoxypyridoxine and isoniazid, have produced convulsions.[44,45] A defect in the ability of the neuron to handle amino acids, particularly glutamic acid and glutamine, may be involved.[46] It appears likely that this metabolic abnormality affects the Krebs energy cycle adversely, since vitamin B_6 functions in a glutamic acid-α-ketoglutaric acid interconversion.

Vilter and associates[47] produced a vitamin B_6-deficiency state in 50 subjects given a metabolic antagonist of vitamin B_6, 4-desoxypyridoxine, for 3 to 22 weeks. These patients developed signs and symptoms with or without a vitamin B_6-deficient diet. Most of the patients became irritable; eight became depressed and sometimes somnolent. Seborrheic dermatitis appeared in 29 patients, involving the nasolabial folds, eyebrows, and area around the eyes. It spread over the face and forehead and down the neck. In several it involved the perineum. In two subjects pellagra-like hyperpigmented lesions developed on the arms and legs or intertrigo appeared in the groin or under the breasts. Glossitis was observed in 14 patients. Cheilosis and angular stomatitis developed in three; conjunctivitis occurred in six patients. Peripheral neuritis was found in 3 patients. Of the 50 subjects, 18 were studied hematologically. Lymphopenia developed regularly in 17 of the 18 persons, but an anemia developing in 7 could not be related to the vitamin B_6-deficiency state. When infections occurred in six, usually in the forms of cystitis or pyelonephritis, there was poor response to antibiotics. After vitamin B_6 was administered, some of the infections responded well. All the above abnormalities were relieved by administration of vitamin B_6 but were not influenced by use of other B-complex vitamins. Linoleic acid relieved the skin lesions only.

This study indicates that vitamin B_6 deficiency induced in human beings by a metabolic antagonist is not very different from the syndrome observed in laboratory animals given a deficient diet. Skin, mucous membranes, and nerve tissues are affected. Lymphocytes are decreased and infections respond poorly, suggesting a reticuloendothelial abnormality which interferes with antibody production. Tryptophan metabolism is abnormal. The skin response to linoleic acid suggests a relationship to essential fatty acid metabolism. It is well known that all these biochemical areas require vitamin B_6 for activation.

Though we recognize that vitamin antagonists often do not reproduce the complete deficiency state but rather may alter one enzyme system maximally, while having little or no effect on another, the clinical picture produced is the closest that investigators have come to vitamin B_6 deficiency in the adult human being.

Isoniazid

Biehl and Vilter [48] were able to prevent the neuritis which occurred in patients who received large doses of isoniazid by giving vitamin B_6 concurrently. Two to three per cent of patients receiving isoniazid in doses of 5 mg. per kilogram of body weight developed this abnormality, whereas forty per cent of patients receiving 20 mg. per kilogram of body weight developed this abnormality. Fifty milligrams of vitamin B_6 was sufficient for prophylaxis. It is known that isoniazid inactivates certain decarboxylase enzyme systems which require pyridoxal phosphate as coenzyme. Increasingly large doses of isoniazid increases the amount of pyridoxal, but not pyridoxic acid, excreted in the urine; xanthurenic acid excretion after tryptophan also increased, but in irregular fashion. For this reason, Vilter and Biehl proposed that isoniazid may couple with pyridoxal to form a pyridoxal-isoniazid hydrazone, thus inactivating enzyme systems dependent on pyridoxal and causing large amounts of a vitamin B_6 compound to appear in the urine. These studies indicate that new drugs may block certain aspects of vitamin metabolism. In the case of isoniazid, only nerve tissue and tryptophan metabolism seems to be affected.

Anemia

Recently Harris and his associates [49] reported the first case of pyridoxine-responsive anemia in a human adult. Up to this time no evidence was available that abnormal hematopoiesis occurs in human beings due to natural or antagonist-induced deficiency. They reported a 35-year-old man with severe normocytic hypochromic anemia unresponsive to the usual hematinic agents. A detailed diet history in this patient showed a vitamin B_6 intake of approximately 3 mg. a day. This amount is greater than the estimated daily requirement. The patient had had anemia since 1942, but prior to use of pyridoxine in 1955, therapy had been unsuccessful. The initial bone marrow was hyperplastic, containing cells of the erythroid series (basophilic normoblasts) which were not forming hemoglobin. All laboratory tests for hemolytic states were negative. Hemoglobin electrophoresis was normal. Erythrocyte autosurvival time done by the Cr^{51} method did reveal a significant decrease in erythrocyte survival. The patient had clinical features of nocturia, tibial pain, and swelling of his feet, all of which disappeared with the use of vitamin B_6. Prior to vitamin B_6 therapy, iron metabolism was abnormal, with a serum iron level of 170μg/100 cc. of blood, total iron-binding capacity of 184μg/100 cc., and an iron-binding protein saturation of 92%. After therapy the serum iron level fell to 67μg/100 cc., total iron-binding capacity was 214μg/100 cc., and the saturation fell to 31%. Reticulocyte response was prompt, with a peak of 50.8%. On cessation of vitamin B_6 therapy the hematocrit fell from 49% to 27% and hemoglobin from 13 to 7.8 gm/100 cc. over a two-month period. Since a diet deficient in vitamin B_6 was not found, it was assumed that either a metabolic block may have existed which increased the requirement of the hematopoietic tissues for this vitamin or that an absorptive defect was present.

Vitamin B_6 and Pregnancy

For many years pyridoxine has been used empirically in women with vomiting of pregnancy. A physiologic change in B_6 metabolism during pregnancy was demonstrated by Wachstein and Gudaitis,[50] who showed that women in the second and third trimesters of pregnancy given a test dose of tryptophan excreted abnormally large amounts of xanthurenic acid in the urine compared with nonpregnant women. This abnormality was quickly relieved by pyridoxine. Wachstein and Gudaitis [51] studied urinary excretion of 4-pyridoxic acid, the metabolic end-product of the entire B_6 complex. It was found to be 20% lower in pregnant women than in normals following a test load of 25 mg. of pyridoxal hydrochloride orally. These observations have

been used to support the thesis that an absolute deficiency of vitamin B_6 exists in pregnant women due to dietary inadequacy, demand of the fetus, and vomiting. However, there are no reports of clinical signs and symptoms in pregnant women which can be ascribed to vitamin B_6 deficiency.

Thirty-one women were studied in our laboratory during the first four days post partum.[52] The amount of xanthurenic acid excreted in the urine after tryptophan load was determined. Vitamin B_6 levels were determined in blood, urine, and skin. Blood levels, as in normal persons, were so low they could not be interpreted. Throughout the pregnancy and the time of the study the patients were taking a normal diet without vitamin B_6 supplements. None of the women had complications of pregnancy or had clinical evidence of vitamin B_6 deficiency.

Determinations were made of urine xanthurenic acid levels and urine and skin vitamin B_6 levels in 9 of these women, urine xanthurenic acid levels and skin vitamin B_6 levels in 14, and urine xanthurenic acid and vitamin B_6 levels in 8.

Of the 31 patients, 22, or 71%, had elevation of xanthurenic levels above 50 mg/24 hours, whereas 32% of a nonpregnant control group of young women from the gynecological service had values above this figure.

Of the 22 patients with high xanthurenic levels, 18 were studied for the concentration of vitamin B_6 in the skin. Fifteen had normal and three had elevated levels. The normal range for vitamin B_6 in the skin as determined in this laboratory for nonpregnant women is 0 to 1.6μg. per gram of dry skin weight. None of the postpartum women studied fell into the lowest range of the normal group. The majority had values from 0.2μg. to 1.6μg. per gram. Three patients had elevated levels over 1.6μg. per gram.

Ten patients were studied for vitamin B_6 excretion in the urine. Four were in the normal range, and six had levels elevated over 0.3 mg/24 hours. None of the women in the postpartum group had values below the normal range of 0.1 to 0.3 mg/24 hours. All of the patients with increased levels of vitamin B_6 in the skin had elevated urine vitamin B_6 levels, demonstrating a correlation between the elevated tissue levels, as represented by skin, and elevated vitamin B_6 levels in the urine.

The tryptophan-xanthurenic acid data substantiate an abnormality in B_6 metabolism during pregnancy. The results of urine and tissue studies do not present any evidence for vitamin B_6 depletion of tissues during pregnancy. Rather, the normal or elevated skin and urine vitamin B_6 levels of postpartum women in the presence of abnormal xanthurenic acid excretion suggests the hypothesis that there is an undefined metabolic defect which increases the need of the pregnant women for vitamin B_6. A relative rather than an absolute deficiency of vitamin B_6 appears to exist.

Antibody Formation

Animal studies have shown that vitamin B_6-deficiency states impair antibody formation. Stoerk[25] found a 95% reduction in specific antibody formation in vitamin B_6-deficient rats given intraperitoneal injections of washed sheep erythrocytes. Axelrod[26] also found severe depression of antibody response to stimulation by injection of human erythrocytes in pyridoxine-deficient rats. The total leukocyte counts in his animals were decreased with lowered lymphocyte counts in the deficiency state. A correlation, however, between the number of circulating lymphocytes and the ability to produce antibodies could not be demonstrated. Serum protein electrophoretic patterns were also unaffected. Vilter and Mueller,[47] who produced pyridoxine deficiency in adult human beings by the metabolic antagonist 4-desoxypyridoxine, also demonstrated depression of total lymphocyte counts. Some of their patients developed resistant urinary infections responding to antibiotics only after the addition of pyri-

Antibody Formation in an Adult Patient Given a Vitamin B_6 Antagonist, 4-Desoxypyridoxine and in His Control *

Wk.	Urine Xanthurenic Acid, Mg./24 Hr.	Total Serum Protein, Gm./100 Cc.	Serum Protein Electrophoresis, %					WBC	Total Lymphocytes	Erythrocyte Agglutination Titers †		Typhoid Titers †		Bone Marrow, Plasma Cells Per 1,000 WBC
			Alb.	α_1	α_2	β	γ			1:A	1:B	1:O	1:H	
1	--	7.2	58.2	1.3	10.2	15.9	14.6	6,300	2,016	8	4	80	0	
	--	(7.0)	(58.7)	(5.3)	(12.1)	(10.2)	(13.6)	(5,200)	(1,040)	—	(0)	(0)	(0)	
2 ‡	33	7.55	52.0	5.7	13.0	12.2	17.1	10,200	2,652	8	4	80	0	
	(50)	(6.2)	(41.0)	(9.4)	(14.5)	(15.4)	(19.7)	(7,050)	(1,410)	—	(2)	(0)	(0)	
3	123	7.8	51.9	6.4	10.7	15.0	16.1	8,000	1,520	—	—	—	—	
	(13)	(6.45)	(47.5)	(8.4)	(15.4)	(14.0)	(14.7)	(8,250)	(1,567)	—	—	—	—	
4	175	7.4	46.9	6.6	12.1	14.4	19.9	6,300	1,260	—	—	—	—	
	(17)	(6.8)	(52.4)	(8.4)	(11.2)	(11.9)	(16.1)	(5,900)	(1,357)	—	—	—	—	
5	233	7.95	56.8	2.6	10.3	14.8	15.5	5,550	832	—	—	—	—	
	(32)	(6.6)	(53.1)	(7.0)	(11.9)	(13.3)	(14.7)	(7,700)	(1,694)	—	—	—	—	
6 ‖	260	7.6	53.9	5.5	9.3	17.4	14.0	5,950	535	8	4	80	0	20
	(35)	(6.6)	(50.5)	(7.4)	(12.1)	(14.7)	(15.3)	(9,000)	(1,080)	—	(2)	(0)	(0)	(9)
7 §	269	7.7	54.4	5.4	8.2	15.7	16.5	8,000	1,360	16	16	80	0	
	(20)	(7.4)	(48.8)	(6.6)	(14.6)	(11.3)	(18.8)	(6,300)	(1,575)	—	(128)	(10)	(0)	
8	245	8.3	56.4	—	9.1	15.6	18.9	8,750	1,487	2×10^{12}	5×10^{11}	40	10	25
	(21)	(6.7)	(46.6)	(6.1)	(12.2)	(14.5)	(20.6)	(7,250)	(2,247)	—	(2×10^{9})	(320)	(10)	(10)
9	198	7.9	48.2	2.7	10.8	17.6	20.7	7,000	1,470	2×10^{21}	5×10^{20}	40	0	
	(12)	(6.6)	(42.9)	(5.0)	(14.3)	(12.9)	(25.0)	(7,500)	(2,325)	—	(5×10^{14})	(80)	(10)	
10	223	7.7	48.0	6.0	10.5	16.5	19.0	6,600	1,584	5×10^{17}	5×10^{17}	40	0	
	(3)	(6.8)	(45.4)	(8.7)	(9.9)	(14.5)	(21.5)	(6,000)	(1,980)	—	(5×10^{11})	(80)	(20)	
11 ¶	251	7.3	45.5	5.5	10.5	16.8	21.8	—	—	8×10^{13}	8×10^{13}	20	0	
	(28)	(6.8)	(40.9)	(7.8)	(15.6)	(16.5)	(19.1)			—	(5×10^{5})	(40)	(20)	
12	--	7.8	47.7	5.9	11.3	18.0	17.1	—	—	2×10^{13}	2×10^{13}	20	0	
		(6.4)	(43.2)	(8.3)	(13.0)	(16.6)	(18.9)			—	(2×10^{4})	(40)	(20)	
13	--	8.0	43.3	5.3	14.3	17.7	19.7	—	—	9×10^{12}	4×10^{13}	20	0	
	--	(6.5)	(45.8)	(7.4)	(12.5)	(17.1)	(17.1)			—	(1×10^{5})	(40)	(0)	
14	--	8.0	55.4	5.5	10.4	13.9	14.9	—	—	2×10^{9}	2×10^{9}	20	0	
		(6.0)	(55.4)	(5.7)	(10.8)	(14.0)	(14.0)			—	(1×10^{5})	(40)	(0)	
15 ‡ ¶	11	8.0	52.1	5.0	9.6	14.6	18.7	6,750	2,362	5×10^{8}	5×10^{8}	20	0	
	(8)	(8.1)	(50.8)	(7.0)	(13.5)	(13.5)	(15.1)	(4,400)	(1,628)	—	(512)	(20)	(0)	

* Numbers in parentheses refer to the control. Patient had type O, control, Type A blood. Fresh pipette used between each dilution.
† Concentration of titers expressed as the reciprocal of twofold serial dilutions of serum.
‡ 4-Desoxypyridoxine, 300 mg. a day orally from 2d thru the 15th week. Placebo in the control. See text for deficiency manifestations.
§ 0.5 cc. typhoid vaccine given s. c. 1 wk. apart to both subjects.
‖ 1 cc. A and/or B blood group substance given on 2 successive days s. c. to both subjects.
¶ 150 mg. oral pyridoxine started in both subjects from 11th through 15th wk.

doxine, suggesting a possible depression of response to infection.

Studies in our laboratory on antibody formation have been carried out recently in human subjects made vitamin B_6-deficient by 4-desoxypyridoxine. Four patients and four comparable control subjects from a chronic-disease hospital were used. The entire group had such medical conditions as generalized arteriosclerosis, hypertensive heart disease, cerebral vascular disease, multiple sclerosis, rheumatoid arthritis, diabetes mellitus, and amyotrophic lateral sclerosis. None had clinical evidence of vitamin deficiencies prior to this experiment, and all were maintained with a regular diet containing 2 mg. of vitamin B_6 per 24 hours. After both clinical evidence and laboratory evidence of vitamin B_6 deficiency were produced, all were given typhoid vaccine and A and/or B blood-group substances † parenterally. Urine xanthurenic acid output after a load with tryptophan, total leukocyte and lymphocyte counts, serum proteins with electrophoretic patterns, bone marrow plasma-cell counts, and serum antibody titers were closely followed through the 11- to 15-week experimental period (Table).‡

The control patients were given placebo tablets, while the others were given 300 mg. of 4-desoxypyridoxine per day by mouth. Typical skin and mucous membrane lesions as previously produced by Vilter and Mueller occurred. Mild sensory peripheral neuritis developed in two patients. Electroencephalograms, obtained before and during the height of the deficiency states, were not altered by the metabolic antagonist, and convulsions were not observed. Total lymphocyte counts dropped in patients receiving 4-desoxypyridoxine, only to show an apparent rise after the antigens were injected (Table). Anemia was not produced in this small group of patients. Appreciable differences in height of antibody titers were not observed between the patients and the controls (Fig. 3). Excellent A and B antibody titer rises were demonstrated in all the eight subjects. Typhoid antibody rises were not striking in most of the patients. After eight weeks, 150 mg. of pyridoxine was given orally to two patients who were receiving 4-desoxypyridoxine in an attempt to demonstrate possible suboptimal antibody response during the deficiency state. This was not observed (Fig. 3).

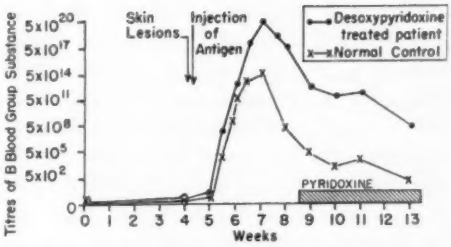

Fig. 3.—Antibody production in a patient treated with desoxypyridoxine for 13 weeks and in a normal control. Concentration of titers is expressed as the reciprocal of twofold serial dilutions of serum.

These observations suggest two possibilities: that 4-desoxypyridoxine is an incomplete antagonist or, in the doses administered, that it does not sufficiently affect the formation of antibody globulin to induce measurable changes in the blood.

Seborrheic Dermatitis

Patients made deficient in vitamin B_6 by the use of the metabolic antagonist, 4-desoxypyridoxine, develop skin lesions quite similar to the natural form of seborrheic dermatitis. Schreiner and associates,[53] in our laboratory, observed that many patients with the siccus type of seborrheic dermatitis responded to local application of pyridoxine but not to oral or parenteral administration of this vitamin. This observation was not confirmed by Effersøe.[54] Recently we have repeated this work with a double-blind study. A total of 59 patients from a state mental institution were used, each chosen for classical seborrheic dermatitis of face and scalp.

† Blood-group-specific Substances A and B were purchased from the Knickerbocker Blood Bank, New York.

‡ Miss Betty Matuska provided technical assistance during the antibody studies.

In each of three groups of patients used, approximately one-half received a cream containing 1% vitamin B_6 and the other half, only the bland cream base (Neobase and Vanibase). The experiment was conducted over a three-week period, and during this study neither the nurses nor the doctors who participated in the work were aware of the contents of the material used.

The cream was applied three times a day, each time to the same side of the scalp and face involved. Initially the severity of these skin lesions was graded on an arbitrary scale. Thereafter each patient was examined at least once a week and was regraded.

A total of 28 patients received the 1% vitamin B_6 cream, while 31 received the placebo cream alone. Of the 28 patients treated with vitamin B_6 topically, 19, or 70%, showed a good response. In the control group, 70% also were found to have a similar response. It was noted that remissions often occurred only after three weeks of topical application of either cream. This observation probably explains the error in the previous experiment. At that time, the bland cream was applied for only two weeks. Following immediately thereafter, applications of the vitamin B_6 cream were begun to the same skin areas. Lesions began to clear within a week after vitamin B_6 cream was applied. It is apparent now that the result was due to the bland cream alone. However, five subjects with seborrhea received placebo cream on one side while vitamin B_6 cream was applied to the other. In these patients, the skin lesions on the side treated with vitamin B_6 cleared rapidly (in four to seven days), while the other side was unaffected or became worse temporarily. These observations cannot be explained in any other way than that vitamin B_6 had some beneficial effect on seborrheic skin.

Skin levels of vitamin B_6 were studied in normal persons after topical vitamin B_6 was used with and without the addition of intramuscular vitamin B_6. Normal skin levels in 19 subjects ranged from 0 to 1.6μg. per gram of skin. Five persons received local applications of 1% vitamin B_6 cream for 2 to 11 days. Four patients received 600 mg. of vitamin B_6 intramuscularly a day for three to seven days in addition to the local application of vitamin B_6. Six patients received the vitamin B_6 intramuscularly alone. Skin biopsy specimens were then obtained after the areas from which the specimens were to be taken had been scrubbed three times. These were analyzed for vitamin B_6 levels. Five to thirty times as much vitamin B_6 was found accumulated in the skin after application of the cream alone as could be found when the vitamin was given intramuscularly alone. The parenteral form of vitamin B_6 in these experiments was not absorbed into the skin and did not alter the skin levels of vitamin B_6.

Summary and Conclusions

Deranged vitamin B_6 metabolism induces lesions in the skin, mucous membranes, nerve tissue, and hematologic and lymphatic systems. The metabolism of tryptophan, protein, and fatty acids may be affected adversely by this deficiency. Specific metabolic abnormalities may increase the need for vitamin B_6, and drugs may cause toxic manifestations by interfering with the function of this vitamin.

Human beings in whom a vitamin B_6 deficiency state was induced by the antagonist 4-desoxypyridoxine formed antibodies to A and B blood-group substances and to typhoid vaccine as efficiently as did well-nourished persons.

Gastroenteric tract organisms are not an important source of vitamin B_6 in human beings. When the numbers of these organisms are reduced by poorly absorbed antibiotic drugs, the excretion of vitamin B_6 in the urine does not change.

Seventy per cent of women who are pregnant or are in the immediate postpartum period excrete abnormally large amounts of xanthurenic acid after a tryptophan load. Nevertheless, women with this abnormality

of tryptophan metabolism have normal or slightly elevated vitamin B_6 levels in urine and skin. These results suggest that any vitamin B_6 deficiency is relative rather than absolute.

The siccus type of seborrheic dermatitis, reported previously to respond favorably when a cream containing 1% vitamin B_6 is applied, responds just as well when the cream base alone is applied for three weeks, as demonstrated by a double-blind study. However, five persons with symmetrical facial seborrhea treated with pyridoxine cream on one side and placebo cream on the other improved in less than a week on the side to which the pyridoxine cream was applied. The other side was unchanged or worsened, suggesting that in some persons an inadequate amount of vitamin B_6 in the skin may contribute to the development of the seborrheic lesions.

Vitamin B_6 is a very important substance in the diet of human beings. Its chemical and biological activities suggest that it may have an important bearing on the arteriosclerosis problem and on the function of the central and peripheral nervous system, the bone marrow, the skin, and the mucous membranes.

Dr. Nathaniel S. Ritter, of the Merck & Co., Inc., Rahway, N. J., supplied the pyridoxine, 4-desoxypyridoxine, and DL-tryptophan used in our studies.

Department of Internal Medicine, Cincinnati General Hospital 3231 Burnet Ave. (29) (Dr. Vilter).

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Correspondence

ESSENTIAL FATTY ACIDS AND ATHEROSCLEROSIS

To the Editor:—It is with great interest that I read in the July, 1957, issue of your ARCHIVES the article (by G. V. Mann) "Essential Fatty Acids and Atherosclerosis," especially his concluding remark on the fact that " . . . we obscure this problem by equating atherosclerosis and coronary thrombosis." As a matter of fact, I hope you will find interesting the following observations: While on a tour through Africa, I noticed (and this is a well-established and universally known fact) that coronary sclerosis is extremely rare among the native (Negro) population in Central and South Africa, whose intake of fats is rather very low, but common (and in some places even very common) among the whites—whose food habits are, more or less, like our own habits in the United States. Now all this is well known. But what seems to me rather little known is the fact—brought to my attention by leading internists in these countries—that cerebral and peripheral vascular atheromatosis is very very frequent among the "natives" and much more fatal than among the whites—thus pointing to a striking lack of parallelism among the consequences (if any) of a fat-rich diet insofar as its impact on vessels in different organic systems is concerned—thus lending support to Dr. Mann's concluding remarks. These facts should perhaps instill a certain amount of scepticism into those hyperenergetic foes of high-fat diets, whom we see so often.

Incidentally, and even though the following remark is pertinent to an entirely different subject: the mulatto population in some of these countries is famous for the tremendously large proportion of heavy smokers; its rate of lung cancer is ostensibly the world's highest.

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IDIOPATHIC CLUBBING AND HYPERTROPHIC OSTEOARTHROPATHY

To the Editor:—This letter is in explanation of an error in our paper "Idiopathic Clubbing and Hypertrophic Osteoarthropathy," which appeared in the March, 1956, issue of the ARCHIVES.

Since publication of our paper our attention has been drawn to discrepancies in the data. We have reviewed these with the laboratory which performed the tests, the Cardiovascular Service at Marquette University. We wish to report that the value given for pulmonary artery pressure is in error and represents a typographical error and that the value given for right ventricular pressure is correct and represents the true value.

A second discrepancy is not so easily explained and relates to values given for resting arterial oxygen saturation (90%) and arterial oxygen saturation after inhaling 100% oxygen (94%). These figures were presented as normal and actually represent some reduction. Our explanation is that the data were presented to Dr. Hrowat and me as being within normal range for this laboratory, and our paper written under this premise. Since then, we have learned that the data were discussed at the laboratory and at a weekly heart conference composed of 7 to 10 cardiologists and that the majority opinion was that the abnormal oxygen saturation studies were probably in error in view of all the other normal findings; it was decided that the case be presented as one of idiopathic clubbing.

It is our opinion that the case is actually one of idiopathic clubbing, and we agree with the members of the heart conference that the data represent normal findings other than the saturation studies, which are therefore probably in error. In retrospect, we feel that oxygen saturation studies should have been repeated in order to confirm or refute the original findings.

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Books

EDITED BY WILLIAM B. BEAN, M.D.*

Science Looks at Smoking: A New Inquiry into the Effects of Smoking on Your Health. By Eric Northrup. Price, $3. Pp. 190. Coward-McCann, Inc., 210 Madison Ave., New York 16, 1957.

Several years ago I introduced what has now become our established pattern of signing book reviews with a blast at Ochsner's weird exhibit of contemporary witchcraft as it applies to tobacco smoking. Northrup's book, written in a breezy, informal style, is destined to give great solace to smokers and those who gain a livelihood in the tobacco industry. It provides a counterpoise to Ochsner's book by being immoderate in its moderation. It does present both sides of the picture, however, and emphasizes again and again the probability that many more sides of this problem exist somewhat out of reach and beyond present knowing.

The future may look back on our times as remarkable for great naïvete about science and research. Science has come to be deified by the man in the street as sign and symbol of this age. Like many past deifications the exploiters and devotees who have brought about the apotheosis of science have had only the haziest notion of what it is all about. The average man thinks that the machines, the bubbling retorts, the trance-like expression of benignant wisdom, that vacuous television look of magical inspiration, the great invention by the great inventor, are what science is all about. Nothing could be further from reality. Science is an attitude of mind. It is the intelligent employment of techniques rather than the technical employment of intelligence. We certainly could not do without the machines of science. Techniques and apparatus without ideas and informed intelligence become the playthings of dilettantes who bestrew medical writings with the dry irrelevancies of myriads of unconnected facts.

Few people who have not invested time and effort in research, clinical research in particular, have any valid conception of the enormous amount of work necessary to establish the simplest fact. The obverse of the coin is the fabulous difficulty of eradicating error; anything said with authority becomes firmly established in men's minds. It is embedded in the matrix of their prejudices and survives with a perversity which Wilfred Trotter has described as "the mysterious viability of the false."

A point which seems to confuse not only laymen but many excellent scientists is the difficult but necessary distinction between observation and experiment. This has an unhappy correlary which must forever remind us that the more exact the experimental design the more abstract it becomes and the more distantly it is separated from the kaleidoscopic hodgepodge of miscellaneous factors which constitute the biological relationships of life.

Northrup reviews a monumental collection of statistics which show a singular parallelism between heavy cigarette smoking and increasing incidence of cancer of the lung. He also points out that a great many other things have increased in parallel, notoriously, the acrid smogs and vapors of our industrial communities. It is clearly emphasized that most of the studies directed at evaluating the relationship of smoking and cancer of the lung have advertently or inadvertently left out other factors. If there is any field where multiple causality operates, it is in the genesis and pathogenesis of cancer. Here the poorly developed skills of ecology by their absence or poverty have had important negative effects.

When two variables are examined, the observational method of science enables us to determine whether a correlation exists or not. It can tell how close the correlation is, and whether it has statistical significance, and that it was unlikely to have occurred but by pure chance. The temporal relationships must be determined with minute care and compulsive accuracy because of the human tendency to *post hoc* reasoning. Parallelism is more significant if secular trends fluctuate up and down rather than merely continuing in a linear trend. But

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correlation, even if it is infinitely significant from the statistical point of view, permits us to make no judgment about causality with certainty.

In the clinical fields of medicine in nearly all circumstances causality must be established by experiment. In a particular patient, nature may perform an experiment for us and reveal a truth which we could get in no other way. With the exception of wars, famines, violence, and concentration camps, which by their nature reduce any opportunity for objectivity, protracted experiments involving the manipulation of masses of people have not been done. Nevertheless, if we wish to get evidence about a putative causal relationship of smoking to cancer of the lung the most information will come from an experimental manipulation of population groups. A hitherto unreached degree of control over the subjects must be achieved. It is idle to enumerate all the factors which must be controlled, but age, sex, genetic strain, climate, environmental poisons, nutritional state, past history of disease, occupation, and dozens of others must be taken into consideration. A significantly large population group must be brought under control for a definite experiment. Keeping all factors as nearly constant as may be, heavy cigarette smoking must be the variable. This is done by dividing the group of naturally heavy smokers into a nonsmoking group while leaving the remainder as heavy smokers. The opposite aspect of the experiment would be to convert a certain number of the nonsmokers into heavy smokers. If these two new groups, the reformed heavy smokers and the newly made heavy smokers, persisted for years in their assigned ways, there would be four groups for study. If it should turn out that the original nonsmokers who became heavy smokers and were followed for a long enough time developed an increased incidence of cancer of the lung, the evidence would be very much better but not in itself completely conclusive. Likewise, if those who had been heavy smokers became compulsive or forced nonsmokers and had a lower incidence of cancer of the lung than their original comrades-in-smoking who continued to smoke heavily, the evidence would be still more convincing. In the last analysis, however, no amount of correlation will be satisfactory unless scientists can detect mechanisms to explain the processes they have observed. It is altogether unlikely that an experiment of the kind I have outlined will ever be done in large enough groups to provide the evidence. A scientific solution of the problem is not possible when we have only the testimony of observed experience.

The virtue of Northrup's book is that the question is looked at pro and con. The light and airy style, a sort of cheerful, breathless Jack-in-the-corner, look-Mommy-no-hands approach may trouble medical readers who are used to prose ranging from turgid to arid. The real weakness of the book is a failure to take up the serious question of the nature of scientific proof and the inevitable failure of observation as a solution to this question.

Even though proof, final and absolute, is far away or even impossible, circumstantial evidence may be enough for action if not conviction. Recently the Medical Research Council of England expressed the view that a major part of the great increase in deaths from cancer of the lung among men during the last twenty-five years is a result of heavy smoking, particularly heavy cigarette smoking. Their conclusions were based on two types of studies. One has been to interrogate people who have cancer of the lung to find out how many of them have been heavy smokers for a long time and, secondly, to learn the smoking habits of a large group in a specific population and ascertain the cause of death in those who die subsequently. Both types of investigation yielded the same results. There was a higher mortality from cancer of the lung in smokers than in nonsmokers. The mortality was higher in heavy smokers than in light smokers. The mortality was higher in cigarette smokers than in pipe smokers, and, finally, it was higher in those who continued to smoke than in those who had given it up. The Medical Council in summary concludes that the most reasonable interpretation of this evidence is that the relationship is one of cause and effect. Thus, lacking proof, we may base our action on our beliefs, remembering that we have halted far short of proof.

As with most questions about which violent passions churn, we shall follow the school of belief about smoking which appeals most to our own practices, to our established prejudices with or without regard to what we have been taught, and to our own peculiar customs in witch-hunting for which mankind seems to have an eternally open season. To man's inherent taste for cruelty to man we will continue to add the inability of many disciplined and intelligent persons to think soundly about scientific matters or to realize how far from the truth are so many of our fondest beliefs.

WILLIAM B. BEAN, M.D.

Peripheral Circulation in Health and Disease. By Walter Redisch, M.D., F.A.C.P., and Francisco F. Tangco, B.S., M.D. Price, $7.75. Pp. 154, with 29 illustrations. Grune & Stratton, Inc., 381 Fourth Ave., New York 16; 99 Great Russell St., London, W. C. 1, 1957.

This monograph is a review and an evaluation of the information currently available concerning peripheral vascular disorders. The anatomy and physiology of the peripheral vascular tree and methods of evaluating blood flow are discussed. The alterations in blood flow occurring with specific pathological processes are described. Compensatory physiological processes are discussed. A comprehensive review of the current medical and surgical measures employed in attempting to correct these peripheral vascular functional and anatomical abnormalities, as well as general vascular disorders, is included. In the final part of the book the methods employed and results and significance of recent researches concerned with muscle vasculature are reported. The evidence presented in this interesting section suggests that classic anatomical and physiological concepts of the blood supply of muscles is in need of revision. Strong support for the proposed changes is provided by the excellent photographs of musculograms, stereoarteriograms, and microradiographs.

The organization of this doubly indexed book into parts and chapters, the latter sometimes with vague headings, necessitates a considerable amount of time for the reader before he can discover the scope of the book or find those parts in which he has interest. The text is supported by an extensive bibliography, occasionally deficient in not including recent important references. A valuable service has been done for the interested physician in sifting and selecting, summarizing and evaluating, the large volume of information currently available concerning peripheral vascular disorders. House officers, medical students, general practitioners, internists, surgeons, anatomists, and physiologists will find this book useful, in the order listed. It is primarily recommended to clinicians, although those investigators primarily interested in muscle physiology will find the section on muscle blood supply of considerable interest.

JOSEPH A. BUCKWALTER, M.D.

Essentials of Fluid Balance. By D. A. K. Black. Price, $3.75. Pp. 123, with 6 illustrations. Charles C Thomas, Publisher, 301-327 E. Lawrence Ave., Springfield, Ill., 1957.

The little Black book has a pleasant sprinkling of dry humor. It is apparent that the author is well read in the subject, but even more important, he has taken time to coordinate his own impressions. He dwells on aspects which have seemed most interesting to him. In spite of this, or perhaps because of it, he covers most of the subject. For those who have read a dozen of these little books here is one that can still be read with pleasure. He dwells on points that all of us find difficult. He presents the subject concisely and simply and directs the reader to the best source material available for additional information.

EDWARD E. MASON, M.D.

Fluid and Electrolytes in Practice. Second Edition. By Harry Stratland. Price, $6. Pp. 229, with 31 illustrations. J. B. Lippincott Company, 227-231 S. 6th St., Philadelphia 5; 2083 Guy St., Montreal, 1957.

There have been over a dozen small books on this subject in the last few years, and now revisions of some of them are appearing. This must mean that there is a tremendous appetite for the subject in a condensed form. Statland has "revised" very little of his book and added three hundred words on salicylate poisoning. The book is fairly good in its theoretical and didactic aspects but could be improved where he attempts to outline treatment of specific patients. The paragraphing, spacing, and sentence structure have been changed a little. An illustration on electrocardiographic changes in hyperkalemia has been reduced in complexity, and another has been added on hypokalemia.

EDWARD E. MASON, M.D.

Irvington House Conference on the Rheumatic Child and His World. Pp. 68. Price, not given. Irvington House, 17 E. 45th St., New York 17, 1957.

The Irvington House Conference on The Rheumatic Child is a comprehensive report concerning the over-all medical and nonmedical services which are maintained for the patient with rheumatic fever. The article is of interest primarily to those who treat children afflicted

with rheumatic fever and is a group discussion of cases representing the opinions of the social worker, doctor, etc. as to the present and future handling of certain problem cases. The session follows the same pattern one would expect regarding patients in a sanitarium or hospital for long-term care, such as tuberculosis, polio, and cerebral palsy.

For the doctor who treats patients with rheumatic fever, the conference is of interest in demonstrating that there is no set dogmatic approach for every patient, but the over-all care must be flexible and adjusted to each "total patient." Teamwork, integration, and cooperation of the social, educational, emotional, and medical facts are all necessary for the treatment of the "rheumatic child." In this respect, the medical staff, the social worker, nurse, teacher, and program worker equally share an active part, and the latter are not merely auxiliary services to the medical department.

This presentation of a typical weekly conference will furnish interesting reading to the physician, but the discussion is somewhat prolonged and repetitious and could have been made just as informative by two well-chosen case presentations.

ELOISE E. JOHNSON, M.D.

Ciba Foundation Colloquia on Endocrinology: Volume X. Regulation and Mode of Action of Thyroid Hormones. Edited by G. E. W. Wolstenholme, O.B.E., M.A., M.B., B.Ch., and Elaine C. P. Miller, A.H.-W.C., A.R.I.C. Price, $8.50. Pp. 311, with 114 illustrations. Little, Brown & Company, 34 Beacon St., Boston 6, 1957.

Fifteen conferences devoted to endocrinology have been sponsored by the Ciba Foundation, London, England. During June, 1956, the first colloquium devoted exclusively to the thyroid gland was held. Dr. Rosalind Pitt-Rivers was the chairman. Participants included distinguished thyroidologists from Great Britain, France, New Zealand, Holland, Brazil, Switzerland, and the United States. The deliberations of the colloquium were restricted to the physiologic mechanisms responsible for the regulation of the thyroid gland and to the character and mode of action of thyroid hormones. Volume 10 of the Ciba Foundation Colloquia on Endocrinology is composed of the papers presented, discussions of each, and the general discussions. Author and subject indices, illustrations, and bibliographies are included. Investigators working in these specific fields should all have a copy of this book. Thyroid physiologists will find it a valuable reference source. It will have limited interest for clinical thyroidologists.

JOSEPH A. BUCKWALTER, M.D.

Pediatric Cardiology. By Alexander S. Nadas, M.D., F.A.A.P. Price, $12. Pp. 587, with 343 illustrations. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5; 7 Grape St., Shaftesbury Ave., London, W. C. 2, 1957.

Dr. Nadas describes his text as a "handbook for the pediatrician, the general physician, and the medical student." This volume of 587 pages is more than a handbook, and although no attempt has been made to cover each subject exhaustively, much detailed information has been incorporated. The literature abounds with articles pertaining to pediatric cardiology, but from a practical standpoint it is difficult for many physicians to familiarize themselves with all of the facets of the subject. One of the outstanding contributions of this book is that it has synthesized the clinical and laboratory aspects of pediatric cardiology in a concise and lucid fashion. Although emphasis has been placed on the clinical recognition and management of heart disease in children, hemodynamic and physiological considerations have not been ignored. The book is well balanced and well organized. The text is divided into four parts. Part 1 includes methods of diagnosis, such as the various forms of radiographic examination, electrocardiography, phonocardiography, and cardiac catheterization. The section on electrocardiography very appropriately emphasizes some of the problems peculiar to the interpretations of tracings in children. Part 2 includes chapters on acquired heart disease as well as discussions of cardiac arrhythmias and congestive failure. Part 3 is devoted to congenital heart disease. Part 4 is a short discussion on anesthesia for children with heart disease. This book contains much information which is not found in many pediatric texts or in some textbooks of cardiology. It should be a very useful book for physician, pediatrician, and general physician alike.

E. O. THEILEN, M.D.

The American Fluoridation Experiment. By F. B. Exner, M.D., and G. L. Waldbott, M.D. Edited by James Rorty. Price, $3.75. Pp. 277, with tables. Devin-Adair Company, 23 E. 26th St., New York 10, 1957.

If we had very delicate but inordinately capacious scales with mystical capacities we could estimate the quantity of hatred and hostility extant in the world. Often in the past this has been channeled off into extravagant polemics amongst those who exchanged hostilities and thus were able to discharge some of the evil force of hatred. Scientists, being men, are not impervious to emotions. These may get so far out of control as to vitiate or simply neutralize their best intellectual efforts. In "The American Fluoridation Experiment" is condensed a large measure of invective describing fluoridation as a gigantic plot to poison us all. It is a remarkable example of the testimonial approach toward reality illustrating by bald statement and breathless innuendo what the authors denounce as a similar technique on the part of those on the other side of the fluoride curtain. The problem at issue is relatively simple. As in many scientific problems the amount of evidence required to establish the simplest fact is staggering. Evidence has accumulated from a variety of sources that the introduction of fluorine into drinking water at nontoxic levels is a factor in reducing the incidence of dental caries, but admittedly only one of many factors. The thesis of Exner, Waldbott, and Rorty is that this is a Machiavellian plot aimed at nothing less than poisoning the whole American people. They are able to mix vitriol with antifluoridation propaganda, illustrating with melancholy rigor the inordinate ability of organized emotions and nearly hysterical reactions to ratify ignorance. We see once more the age-old human trend of the ignorant to gull the innocent.

Still it is fortunate that in a democratic society censorship does not suppress even such a passionate book. Its gadfly effect should be to encourage the exponents of fluoridation to reevaluate the evidence. The unfortunate side is that this is an example of the antiscientific attitude masquerading as science. It will be interesting to see whether this paranoid volume does anything more than lend the formality of a printed book to the antiscientific outbreaks which abound in the antifluoridation program. This program depends on the fact that the anti- arguments are easy to understand and follow, whereas those of the proponents of fluoridation are more difficult to grasp. These particular anti- arguments have their basis in some of mankind's most deeply imbedded prejudices and emotions. The fear of poison always haunted primitive man, stimulated mithridates, and has never rested since the activities of the Borgia's. In our land the right of the individual person to poison himself rather than to be poisoned is to exemplify in the flagrant folly of self-medication and the widespread permeation of the man in the street by the potent contents of pills and potions of all sorts. Indeed, some drugs are recommended on grounds of enthusiasm rather than evidence by physicians.

The individual right of someone in a democracy to medicate, drug, and poison himself, an aspect of the do-it-yourself cult, leads from self-diagnosis through self-treatment to the sad climax of self-sacrifice. This permissive situation regarding self-destruction has not hitherto led to any question of the demonstrated efficacy of iodizing salt, the employment of pure-food-and-drug regulations, the development of vast sewage-disposal systems, the provision of germ-free water supply, and other efforts at mass-producing health. Furthermore, the action of the Federal government or its agencies, be it ever so beneficial, inevitably brings howls from members of whatever party or group happens to be out of focus politically at the moment or just in a bad temper or self-medicating their private griping of the guts.

In sum, we have a strong example of the inability of ultraspecialized man to deal with the complexity of the modern world. He reacts to the inevitable unknown with fear or rage. This book is an example of the all-pervasive paranoia which has become a brand of our age.

WILLIAM B. BEAN, M.D.

Physiopathology of the Reticulo-Endothelial System. Edited by B. N. Halpern. Price, $9. Pp. 317, with many (including colored) illustrations. Charles C Thomas, Publisher, 301-327 E. Lawrence Ave., Springfield, Ill., 1957.

An exclusive group of scientists from several countries (Belgium, France, Germany, Great Britain, and the United States) participated in this symposium, which was held in the inspiring atmosphere of an eighteenth-century mansion at Gif-sur-Yvette, undoubtedly in the twentieth century, although this is not indicated in the book. The interests of the participants centered around the reticuloendothelial system (RES), but their approaches ranged from

the physicochemical to the clinical. A colorful French introduction by P. Vallery-Radot is followed by the texts of formal papers and by edited discussions. The papers deal with a wide variety of topics: the morphology of the histiocyte; determination of shape and size of protein particles; effects of cortisone, vitamin B_{12}, and choline on the RES; quantitative studies of phagocytosis by the RES; the role of the RES in the metabolism of exogenous cholesterol and of iron; the function of RES in infections, in the defense against endotoxins, in antibody formation, and in hypersensitivity reactions; pharmacological influences on the RES, and the all-important role of the RES in blood cell destruction under normal and pathological conditions. All papers are in English and are followed by French résumés. Only some of them have English summaries as well. The chairman of the conference, B. N. Halpern, concludes the symposium with a pithy synopsis of the proceedings.

Most of the papers are well written and informative. Some of them, such as the articles of Benacerraf et al. and of Dobson on the "Kinetics of Phagocytosis," are veritable gems. Unfortunately, little can be said in favor of the morphological paper by the *doyen* of French histologists, A. Policard. Author, translator, and editor here joined forces to make a semantic, syntactic, grammatical, and ortho(?)graphic shambles out of a potentially valuable contribution. A few quotations will, I hope, substantiate this judgment without hurting the reader within an inch of his life, as perusal of the whole paper might. "Highly developed rod-shaped mitochondriae with double transversal walls"; "double lamellae with two thick walls, each of which is 70 to 80 Angstroms"; a little farther down, "very thin lamellae less than 0.4," and an expression which would be wonderfully descriptive if applied to the activities of certain political bodies: "dynamic inertia" (of the nucleoplasm).

The discussions are presented in the form of indirect quotations, a device whose usefulness is well known to presidential press secretaries. The foreword deplores the concomitant loss of "much of the verbal crossplay and drama," but judging by less stringently edited discussions in other publications this is good riddance rather than lamentable privation.

N. S. HALMI, M.D.

Management of the Patient with Headache. By Perry S. MacNeal, M.D.; Bernard J. Alpers, M.D., William R. O'Brien, M.D. Price, $3.50. Pp. 145. Lea & Febiger, 600 S. Washington Sq., Philadelphia 6, 1957.

Headache is a common and sometimes unrewarding problem, and a fresh thoughtful analysis is welcome.

The authors have tried to clarify and bring sharp lines of distinction between the various types of headache. The six chapters discuss organic mechanisms, psychological mechanisms, vascular mechanisms of head pain, headaches as a symptom of systemic disease, differential diagnosis and treatment, and diagnostic techniques. An endeavor to simplify always risks drawing lines more clearly than the facts justify; but the authors have achieved their aim, and this volume would form a sound foundation upon which further observations and intelligent experience could build. The scope and approach is both wide and wise. This is praiseworthy but not unexpected, for this monograph comes from three departments: internal medicine, neurology, and psychiatry. Unfortunately, the triple authorship is also reflected by some confusion of discussion. One hundred twenty-eight pages preclude a study in depth as well as breadth, and at times this work sacrifices totality to dogmatism. An account of migraine status might appear in a second edition, for example, and those who do not accept food allergy as the basis of autonomic cephalgia might look for further discussion, for the author's successful therapy of this syndrome may well have other explanations. The print is satisfactory but large, the prose, good.

The personal approach of a broadly trained physician to a limited problem will always be of value in medicine. It may stimulate the lazy and frequently appetizes the curious. This is an excellent introduction to the subject at the student, intern, or resident level.

D. B. STONE

The Year Book of Cancer (1956-1957 Year Book Series). Edited by Randolph Lee Clark Jr., B.S., M.D., M.Sc. (Surgery), D.Sc. (Hon.), and Russell W. Cumley, B.A., M.A., Ph.D. Price, $7.50. Pp. 572, with 210 illustrations. The Year Book Publishers, Inc., 200 E. Illinois St., Chicago 11, 1957.

This initial volume, "The Year Book of Cancer," suggests that this and others to follow will be valuable additions to the Year Book Series. The problems in the preparation of such

a volume are multiple; many scientific disciplines are transected, and the writings on neoplasms, from practical clinical recordings to highly theoretical research, represent a vast literature. Still, the many disciplines and extensive literature fall within the scope of oncology.

The editors and the editorial board section editors are drawn from the University of Texas M. D. Anderson Hospital and Tumor Institute. There is, however, a broader representation through the total of one hundred nineteen editors. Each editor was assigned, by reason of special competence, to one of twenty-five sections. The sections are cleavage groups within the general fields of cancer and include anatomic subdivisions on basic research, chemotherapy, steroid endocrinology, genetics, biostatistics, endocrine tumors, special surgical problems, pediatric surgery, metastatic tumors and late care, virology, leukemias and lymphomas, cytology and pathology, and three sections dealing with ionizing radiation. The editors selected bibliographies of articles (two hundred seven articles are presented), and condensations were prepared by the original author(s). The articles are well abstracted generally, although lack of uniformity in method is apparent. A discriminating selection of two hundred seven articles from the more than four thousand cancer articles appearing in 1956 and 1957 is difficult, but the articles and journals are certainly representative. Some clinical weighting in the selection is apparent, probably reflecting editorial intent.

The format is good. The indices are good. The type is clear, and the illustrations are lucid. The editorial comments reflect the privilege of editors. A final section, on the psychologic aspects of cancer, is worth reading.

This volume is recommended as a valuable acquisition for the physician or scientist interested especially in neoplasms and as an addition of merit for the departmental and institutional library.

ROBERT C. HICKEY, M.D.

Hemorrhagic Diseases. By Armand J. Quick, Ph.D., M.D. Price, $9.50. Pp. 451, with 31 tables. Lea & Febiger, 600 S. Washington Sq., Philadelphia 6, 1957.

As with many publications whose subject material is prone to debate and interpretation, this book will be received with mixed opinions contingent upon the reader's training, experience, and knowledge of the blood-coagulation mechanism. Admittedly, this mechanism is complex; the methods of study of it, at times frustrating, and interpretation of results, often subject to question. The multifacetted terminology, hypotheses, and concepts; the crude reagents employed, and the vagaries of the kinetics of some of the enzyme systems involved account for much of the present-day confusion. Yet, if one compares present-day knowledge with that of just a few years ago, it is immediately apparent that the ratio of knowledge to ignorance has increased impressively.

Dr. Quick's "Hemorrhagic Diseases" admirably fulfills the need for a clear, easily read, concise presentation of a complex subject. By virtue of the author's rich experience and many years of investigative work from the prequantitative era to the present, the subject matter included is presented with a perspective and chronological historical approach which contributes to the reader's comprehension and serves to indicate the progressively increasing knowledge of hemostasis in health and disease. As might be expected from the preface, the author often takes pains to defend his own concepts. At times the presentation is almost autobiographical, extolling, especially, the virtues of his one-stage prothrombin test. Whatever opinion the reader might hold of these features of the book, it must be said that they possess the merit of extensive personal exploration of a difficult subject.

The book is divided into two parts, clinical and laboratory methods. The chapters in Part I in their chronological order are as follows: "The Historical Development of the Concepts of Hemostasis," "The Coagulation of the Blood and Its Role in Hemostasis," "The Hypoprothrombinemic States," "Hemophilia and Hemophilia-like Diseases," "Thrombocytopenic Purpuras," "Pseudohemophilias A and B," "Telangiectasia," "Afibrinogenemia and Hyperheparinemia," and "Venous Thrombosis." A detailed critical analysis of each of these chapters is not within the scope of this review. Each is exceptionally well written—as lucidly, probably, as anything on these subjects has been. The various hemorrhagic disease states are discussed with a decided practical clinical flavor, incorporating diagnosis, mechanism or theories thereof, and treatment. Where applicable, genetic and social implications are discussed in appropriate detail. Current and pertinent references are cited in a generally

well-chosen bibliography at the end of each chapter. There are quibbles and even points to which some "coagulationists" might take rather violent opposition, but from a holistic approach, the presentation is generally sound and informative.

In Part II, there are twenty-five assay procedures for the study and diagnosis of the hemorrhagic diseases. This compilation of tests again represents Dr. Quick's personal "armamentarium" of methodology with which, with few exceptions, he has had long experience. Each procedure is described in detail, stating precisely the equipment and reagents needed and the range of normal control values obtained by him with these methods. One of the exceptions referred to above is a method for the detection of fibrinolysin which "we have proposed but have had no opportunity to test." This might better have been deleted or one or another commonly used procedure substituted. In fact, the subject of fibrinolysis might well have been discussed more thoroughly. One matter on which all can agree, however, is the justifiable emphasis on painstaking technique, with respect not only to performance of the tests but to collection of blood samples, scrupuously clean glassware, etc.

With but few qualifications, this book can be highly recommended to all interested in hemorrhagic diseases and especially to internists, general practitioners, clinical pathologists, and those for whom a blood clot represents an intricate labyrinth of biochemical systems.

JOHN R. CARTER, M.D.

The Patient Speaks: Mother Story Verbatim in Psychoanalysis of Allergic Illness. By Harold A. Abramson, M.D. Price, $3.50. Pp. 239. Vantage Press, 120 W. 31st St., New York, 1956.

I have a dreadful suspicion that many physicians and internists have as much difficulty as I have keeping up with formulations of psychoanalysts and even some psychiatrists. I am sure this is a great misfortune, not only for us physicians but for psychoanalysts too. In this state, I am reluctant to read a book by a psychoanalyst, lest his words and concepts, so far removed from biology, medicine, or scientific proof, enhance rather than abate my confusion. Abramson's book, "The Patient Speaks," is remarkable. It is a series of the outpourings of a patient on a psychiatrist's couch, not just for the psychiatrist and his note-taking, but for a tape recorder from which these excerpts are taken.

It has been said that if any person could but write the intimate and clear details of his life he would have the material for at least one novel. This comes very close to being an autobiography along just such lines. We have the sad tale of a woman who was overwhelmed by her mother, dominated and almost engulfed. Finally she severed the umbilical cord which had not only kept her firmly attached to the mother but seemed to have become wrapped many times around her. It compressed and almost stifled her own independent existence. During the three and one-half years of psychoanalysis a series of flashbacks reveal dramatic episodes and those many apparently inconsequential bits which in the aggregate go to make up existence. There is remarkably little intrustion of the analyst. It must be taken as an unkind comment, but I believe this largely accounts for the considerable excellence of this book. It is more autobiography of patient than jargon of analyst. In any event, we get bit by bit a reconstruction of the patient's life, her eczema, the problems that vexed her, and the various futile efforts at relieving her skin trouble along the orthodox lines of allergy. Whatever the cause-and-effect relations may have been, during the course of the analysis the eczema slowly came under control. It all but vanished as a problem in the patient's life as she finally achieved maturity and was able to live an independent existence, so independent in fact that the relationship of mother and daughter almost became reversed.

The value of such a book will be small or great, depending on one's acceptance of the formulations and interpretations. These questions come to mind, and I ask them, not throwing down a gauntlet but wishing illumination. To begin with, three and one-half years and many hundreds of hours strike me as an inordinate amount of time to have to spend in first excavating the debris from the past and reconstructing it. By its ground rules, this limits psychoanalysis to a few wealthy people. Thus psychoanalysis is basically at odds with the traditional approach of medicine to bring help to those who need rather than restricting it to those who can pay handsomely for it. Secondly, what evidence is there that comparable achievement might not be made by someone who provided sympathy, a listening attentiveness, and other insights without using the formulations of psychoanalytic psychiatry at all? In no field of medicine does the apostolic function occur in so exaggerated a form as in psycho-

analysis. It is the psychoanalyst's mission, avowed or not, to convert the patient to his way of thinking and to his own formulations. Using the restitution of the patient toward normal and his rehabilitation in society as a criterion, this form of therapy is not notably more effective than other forms of psychiatric help. One gets the recurring feeling that much psychoanalysis may be understood as follows along these lines. The analyst uses to the full the therapeutic effect of getting the patient to talk out his problems. As a listener he exploits one of the greatest advantages of the physician. Then the patient has to be instructed in a series of concepts based on Freudian psychoanalytic terminology. This might be analogous to learning a new language. Then, when the new language has been learned, it is possible for the patient and analyst to converse freely in the new terms. To what extent these terms do in fact represent reality and to what extent they are interesting figments of vivid imagination has been so far too elusive a point to decide. Nonetheless, from time to time one gets the uneasy feeling that much of this remarkable and fascinating jargon might be a chimera. Finally, when the patient by transference of affection has come to feel a profound attachment for the psychoanalyst and when the dredging of the past has brought up certain elements which have been repressed, new insights are supposed to make the patient realize why he is in the fix he is in. As far as I can make out, adjustments in the field of personal relations must be made at the expense of someone other than the patient. This makes for great difficulties in the family and social complexes if personal relationships are settled merely from one person's point of view.

Abramson's book is an excellent synopsis of the life of a patient, related with great clarity and growing insight. I suspect his own interpretations of his success. Unless a method less time-consuming can be found, psychoanalysis will remain beyond the reach of most patients because of its expense and because it is almost a full-time occupation and preoccupation of the patient. Until psychoanalysis takes into its Freudian bosom critical investigators, skeptics willing and able to apply to its tenets the rules of evidence within the framework of biology and clinical medicine, it remains aloof. Its essentially *post hoc* philosophy and the lack of technique of control and comparison leave it beyond the pale. If the beliefs of psychoanalytic psychiatry are valid, they should pass the test of clinical experiment. If they are not, it should be viewed as another religion, fascinating but impervious to experiment and not available for proof. We may respect it as religion but as outside the corpus of medicine.

WILLIAM B. BEAN, M.D.

Headache—Diagnosis and Treatment. By Robert E. Ryan, B.S., M.D., M.S., F.A.C.S. Price, $6.75. Pp. 421, with 6 illustrations. The C. V. Mosby Company, 3207 Washington Blvd., St. Louis 3, 1957.

Composing a book about a single symptom involves three procedures: (1) collecting the facts and theories, (2) evaluating them, and (3) presenting them.

Dr. Ryan is an otolaryngologist at the St. Louis University School of Medicine and formerly a Fellow at the Mayo Clinic. He has had considerable experience in the investigation and treatment of headache; he has done original work, especially in the assay of new drugs; he has read extensively. Nearly every detail about this large subject seems to be contained in this volume; the discussions on treatment are particularly full; the chapter summaries are clear and helpful, the references, excellent. A cavil is that Jonathan Hutchinson is said to have described temporal arteritis in 1890, forty-four years before Horton's valuable work. The author has thrown his net widely, and some of the objects brought up seem hardly worthy of examination. In this age of experts, the student may hope that the master will teach not only by giving the facts but also by intelligent discussion, by critical evaluation, and by emphasis in order of importance. The insight of the author might have led him to analyze the material more helpfully. In my opinion, this work would have greater appeal if the presentation were more concise.

This book, a somewhat uneven evaluation of an excellent collection of data, is of much value both for basic reading and for reference.

D. B. STONE, M.D.

A Pictorial History of Medicine. By Otto L. Bettmann, Ph.D. Price, $9.50. Pp. 318, with profuse illustrations. Charles C Thomas, Publisher, 301-327 E. Lawrence Ave., Springfield, Ill., 1956.

No one has ever seriously estimated the influence of the camera on modern medicine or the world at large, nor have there been many thoughtful studies of the role of illustration

in scientific treatises. At a time when reading is largely defunct, we witness the great flourishing of pictorial journals, of which a prominent example is the weekly *Life*. For a bird's-eye view of what might be called a picture gallery of medicine beginning with the most ancient statues, paintings, and bas reliefs and continuing up to modern-day photography, Bettmann's "A Pictorial History of Medicine" is an excellent source book. Anyone who really knows medical history can point out a number of errors, and there are some typographical mistakes. The doctor, as he has appeared in fable and play, in lampoon and satire, in praise and glory, from the dim days of prehistory up to the modern era, is recorded in this handsomely printed volume, which would make an excellent present for physician or laymen, though it will, I think, perhaps vex the scholar and annoy the perfectionist. It may be used effectively as a reference book to encourage undergraduates in pursuit of medical history.

WILLIAM B. BEAN, M.D.

The Year Book of Drug Therapy (1956-1957 Year Book Series). Edited by Harry Beckman, M.D. Price, $6.75. Pp. 514, with many illustrations and tables. The Year Book Publishers, Inc., 200 E. Illinois St., Chicago 11, 1957.

With drug therapy developing by leaps and bounds and when one may be hard put to distinguish fact from fancy and fancy from fantasy, it is well to have the stimulating review and commentary which Beckman has added to the summaries of current work on therapy contained in this "Year Book." At a time when LSD and the tranquilizers, serotonin and the molecular antagonists, and psychopharmacology have such prominence, it is a good thing to be able to get at a critical evaluation of current therapy in the "Year Book." It has now become literally impossible for any physician to keep up in any sensible fashion with the vast flood of drugs which issue forth in such spectacular profusion day by day. Even one book a year is not quite able to keep up with the torrent, but here we have refreshing and highly critical comment about the perplexity presented by the tremendous productivity of the highly competitive drug industry. This book goes a long way toward reducing our problems, even if not solving them.

WILLIAM B. BEAN, M.D.

The Doctor, His Patient, and the Illness. By Michael Balint, M.D. Price, $7.50. Pp. 355, with 1 table. International Universities Press, Inc. 227 W. 13th St., New York 11, 1957.

This book by Balint, with a formidable or pretentious title, turns out to be an extremely interesting analysis of the practice of medicine as it relates to the family doctor. Whether one suspects that the family doctor will disappear as a general practitioner and become a well-trained internist or whether one believes that the foundation of medical practice will continue to be a generalist, a benignant tinker, who does all things for all people, including surgery, obstetrics, and psychiatry, this book will provide useful insights. It deals with the practice of medicine as with a sick patient. It consists of an intimate series of critiques held jointly by practitioners and psychiatrists under the auspices of the Tavistock Clinic for the purpose of discussing specific cases and specific forms of treatments in a series of seminars. General practitioners and psychiatrists confront the actual problems of real patients in medical practice and review their failures and successes in an attempt to get a clearer understanding of what medicine is all about. A large part of the book consists of annotations and actual records of discussions of and about patients whose case histories are abstracted for the reader. Only a few American physicians will be confused by "surgery," "panel list," "registrar," and often words with different meanings for the British and for us. I take strong objection to the statement that most patients want to know the name of their illness—the diagnosis—before they want therapy or something done to alleviate suffering. Perhaps British patients are more sophisticated or more stoical than Americans, but in my experience a patient is either bewildered by the name or definitely not interested, at least until he is relieved of the insistent symptoms of his acute difficulty. In only a few places is the text marred by the mythology of certain nonbiological formulations of the avant-garde (or rear-guard) psychiatrists. I am bewildered by the interpretation of the symbolism of dreams which psychiatrists come up with; and while I admire their imaginative discussions, I sincerely believe that the interpretation of dreams has not advanced much since the days of Joseph in the "Old Testament."

It would be well if every physician could analyze his own habits, customs, traits, and strong and weak points as a self-administered critique along the lines suggested in this book. Some of the things considered are what the patient offers to the doctor and the doctor's responses; elimination of inappropriate diagnoses by the physical examination; the incidence and evaluation of neurotic symptoms; the various levels of diagnosis; the collusion of anonymity among doctors; general practitioner and consultant, and the perpetuation of the teacher-pupil relationship. In the realm of psychotherapy, topics considered are advice and reassurance, how to start, when to stop, the special psychological atmosphere of general practice, and the general practitioner as psychotherapist. In various places we find the general practitioner's resistance to the advice and formulations of psychiatrists. With further discussion and better insight, fair compromises are reached in many areas well off the beaten path from ordinary general practice. I find very little to quarrel with in this extremely thoughtful book, which represents a great advance in doctrine and practice and points hopefully to a closer *rapprochement* between physicians and psychiatrists. It is very refreshing to find a psychiatrist who admits ignorance and the need for research. If more psychiatrists would advance from their defensive posture into this inquiring attitude, they would regain their place of respect in the field of medicine.

WILLIAM B. BEAN, M.D.

Industrial Toxicology. Second edition. Lawrence T. Fairhall, Ph.D. Price, $10. Pp. 376, Williams & Wilkins Company, Mount Royal and Guilford Aves., Baltimore 2, 1957.

The second edition of "Industrial Toxicology" is an even better reference book than the first, which appeared in 1949. In the new edition seventy-nine organic and one hundred eighty inorganic materials are listed, an increase of seven and forty-six listings, respectively. The same format is followed, in which the substance is discussed under the headings of characteristics, uses, toxicity, and analysis. Each is followed by a short pertinent bibliography. The material presented is up to date. It is objectively brief and usable.

The present edition includes appendices that enhance the value of the book. Of particular interest is a table of detoxication end-products. Another compares toxicities of various substances. The nontoxicologist using this book will find the introduction of considerable help in orienting him into the field of industrial toxicology.

The first edition has been a standard reference in the field. A new edition is welcomed. Industrial physicians will find it particularly valuable. It is recommended to any physician whose practice includes patients from industry.

CLYDE BERRY, M.D.

The Year Book of Endocrinology (1956-1957 Year Book Series). Edited by Gilbert S. Gordan, M.D. Price, $6.75. Pp. 377, with 73 illustrations. The Year Book Publishers, Inc., 200 E. Illinois St., Chicago 11, 1957.

"The Year Book of Endocrinology" continues to be one of the best of this series. This "Year Book," with many of its literary brothers, has become essential reading for general physicians or internists who see the average run of patients rather than only patients in a particular branch of a specialty. In addition, it will appeal to endocrinologists for the interesting and useful editorial commentary. This volume is embellished by two excellent essays, one by Albright and Larson, on "Thyroid Hormone Transport," and one on "Recent Developments in Glucagon," by Elrick. This year the thyroid and adrenals get a large share of the space, reflecting the very great activity of research in these fields. A fine example from a useful series.

WILLIAM B. BEAN, M.D.

The Year Book of Pathology and Clinical Pathology (1956-1957 Year Book Series). Edited by William B. Wartman, M.D. Price, $7.00. Pp. 510, with 158 illustrations. The Year Book Publishers, Inc., 200 E. Illinois St., Chicago 11, 1957.

In order to vary the routine Wartman has continued his most interesting discussion of what one might call the etiology if not the pathogenesis of this "Year Book." Last year he took us behind the scenes explaining the process of editing. This year he discusses the problem of the journals which contain the papers of interest to pathologists. One-third of the articles abstracted were from foreign journals, though relatively few were not in English.

The journals abstracted comprise a highly selected series with obvious and admitted bias. It is of some moment to internists that forty-two per cent of the articles abstracted come from journals devoted to the basic sciences and internal medicine, which Wartman ascribes to the great investigative vigor in these areas. The second point of particular interest to editors is the fact that relatively few of the papers abstracted had been published in journals of pathology. As with several other books in the "Year Book" series, the pleasant asides and editorial comments add greatly to the interest and pleasure of reading the book and contain many useful facts. For those hopelessly behind in their reading, this volume will be stimulating and refreshing. The beset internist and practitioner will be aided in keeping up with the mad march of medicine.

WILLIAM B. BEAN, M.D.

Ciba Foundation Symposium on the Chemistry and Biology of Purines. Edited by G. E. W. Wolstenholme, O.B.E., M.A., M.B., B.Ch., and Cecilia M. O'Connor, B.Sc. Price, $9. Pp. 327, with 124 illustrations. Little, Brown & Company, 34 Beacon St., Boston 6, 1957.

This book documents the formal papers and ensuing discussions at the Ciba Foundation Symposium held in London in May, 1956. These deliberations about purine chemistry and metabolism appear to have been an outgrowth of an earlier and successful Ciba Symposium devoted to a related topic, the pteridines. That the study of the chemistry and metabolism of purines is extremely active, even competitive, is seen by the depth and scope of the work, reported from governmental, educational, and industrial laboratories alike. Almost half of the papers are probably outside the interest of all but the organic chemist. Nonetheless they are important for pointing the way toward the synthesis of many other purine analogues of biochemical or therapeutic interest. Certain details presented at this symposium, particularly the contribution by G. R. Greenberg, of Western Reserve, concerning the role of folic acid, are sharpening the picture of purine biosynthesis. The cytotoxic purines were given much attention. Purine nucleoside, a constituent of certain mushrooms, was found by G. B. Brown, of Sloan-Kettering, to be far more toxic than free purine, suggesting that the pharmacological potency of many purine agents might be enhanced by conversion to the nucleosides.

The antimetabolites of the purine family act in diverse ways. R. E. F. Matthews, of Cambridge, observed that some of the 8-azapurines having antitumor activity are incorporated into nucleic acid, perhaps thereby smudging the cellular blueprints for protein synthesis. On the other hand, the antileukemia agent, 6-mercaptopurine, blocks natural purine utilization in nucleic acid, according to Hitching's group at Wellcome Research Laboratories. The only paper in which the medical practitioner might feel at home is the discussion by D. A. G. Galton, of the Chester Beatty Institute, of clinical experiences using 6-mercaptopurine for treatment of acute leukemia.

This book is not light reading, nor is it recommended to those unfamiliar with this or closely allied fields. For the researcher in cancer chemotherapy, clinical or otherwise, it maps one of many trails to be followed in quest of effective antitumor agents.

JOHN P. HUMMEL, Ph.D.

News and Comment

ANNOUNCEMENTS

New Medical Journal for 1958.—The American Rheumatism Association announces the forthcoming publication of a new medical journal, *Arthritis and Rheumatism,* the official journal of the American Rheumatism Association. Grune & Stratton, Inc., New York, publishers of the journals, *Blood, Circulation, Circulation Research, Clinical Research Proceedings,* and *Metabolism,* have been chosen by the Association to publish this new journal, which will appear bimonthly, starting with the January-February issue of 1958. The publication committee of the Association is composed of Drs. Richard H. Freyberg, William H. Kammerer, John Lansbury, Charles Ragan, and Charles L. Short. Dr. William S. Clark will serve as Editor, and the remainder of the editorial board will be announced subsequently.

The journal will cover the field of connective-tissue disorders, in particular rheumatoid arthritis, osteoarthritis, rheumatic fever, gout, the so-called "collagen diseases," and non-articular rheumatism. In their choice of original articles the editors hope to achieve an optimal balance between papers relating clinical experience and those which report pertinent developments in the basic science, such as immunology, biochemistry, pathology, and pharmacology. In addition to original and review articles, special departments will include news and notices, correspondence, editorials, progress reports, and book reviews. The contents of the journal should interest not only the specialist in rheumatic diseases but also the internist, orthopedic surgeon, research worker, and all practitioners with a special interest in these diseases.

Special Fellowship in Medical Neoplasia.—Memorial Center for Cancer and Allied Diseases is a training center affiliated with Cornell University Medical College. Specializing in cancer and allied diseases, it participates actively in teaching at both undergraduate and graduate levels. It has a fully approved residency program in internal medicine and, in addition, offers special fellowships for study to a limited number of graduate physicians.

I. Scope and Purposes.—A. To offer the physician trained in internal medicine the opportunities available at Memorial Center to study the natural history, diagnosis, complications, pathogenesis and pathologic physiology, pathology, and treatment of cancer, with particular emphasis on the leukemias, lymphomas, and allied diseases.

B. To undertake an active supervised clinical investigation of interest to the service and the fellow in the field of medical neoplasia (nonsurgical cancer and palliative therapy of patients with malignant tumors).

II. Outline of Activities of the Fellow.—A. The clinical or clinical and laboratory study of an approved or assigned problem under reasonable guidance and supervision. The study is to be carried out at Memorial Center under the aegis of the Medical Neoplasia Service of the Department of Medicine. Available for clinical investigation are hospital records of 40 years, plus an active service consisting of approximately 40 beds in the Memorial Center and two outpatient clinics in which an average of 70 patients are seen weekly.

B. Attendance at a weekly service teaching conference.

C. Periods of assignment to various surgical clinics and conferences, cancer chemotherapy, radiochemistry, radiophysics, and radiotherapy.

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E. Observation of the role of the medical consultant to patients undergoing radical surgery for cancer.

F. An unique opportunity to see and study the management of large numbers of patients with lymphomas, leukemias, and allied diseases.

III. Prerequisites for Appointment to Fellowship.—A. Candidates must be graduates of recognized A. M. A.-approved medical schools and must have completed or be in process

of completing two years of postgraduate training in internal medicine in addition to one year of internship. Such training may be formal residency or its equivalent.

B. Candidates must be of the highest integrity and moral character and have an expressed interest, both academic and clinical, in the study of malignant neoplastic diseases.

IV. Financial Support.—A. The salary stipend is $6000 per annum, without maintenance.

B. The fellowship appointment is for one year, normally beginning July first, renewable for one or two years for select persons who develop a special interest in some problem.

Applicants should apply in writing to Lloyd F. Craver, M.D., Chief, Medical Neoplasia Service, Memorial Center for Cancer and Allied Diseases, 444 E. 68th St., New York 21.

Symposium on Cancer Research.—The Twelfth Annual Symposium on Fundamental Cancer Research will be held March 6, 7, and 8, 1958, at The University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Texas.

The topic for the Symposium will be "Radiation Biology and Cancer." The first day will be devoted to papers from the staff at M. D. Anderson Hospital which relate to the general symposium subject. The final two days of the program will consist of papers presented by recognized authorities in radiation research.

Chairmen for the radiation biology sessions include Titus C. Evans, Radiation Research Laboratory, State University of Iowa College of Medicine; Jacob Furth, Pathology Department, Children's Cancer Research Foundation, Inc., Boston, and Henry S. Kaplan, Radiology Department, Stanford University School of Medicine, San Francisco. General chairman of the Symposium is Warren K. Sinclair, chief physicist at The University of Texas M. D. Anderson Hospital and Tumor Institute.

International Society of Hematology.—The Seventh Congress of the International Society of Hematology will be held in Rome, at the Plazzo dei Congressi, EUR, on Sept. 7 to 13, 1958.

The official languages of the Congress will be English, French, German, Italian, and Spanish.

Preliminary program: (1) Immunohematology; (2) Hemorrhagic Disorders; (3) Leukemia; (4) Anemia; (5) Nucleonics; (6) The Spleen and the Reticulo-Endothelial System.

For further information write to Segreteria del VII Congresso Internazionale di Ematologia, Istituto di Patologia Medica, Policlinico Umberto I, ROMA (Italia).

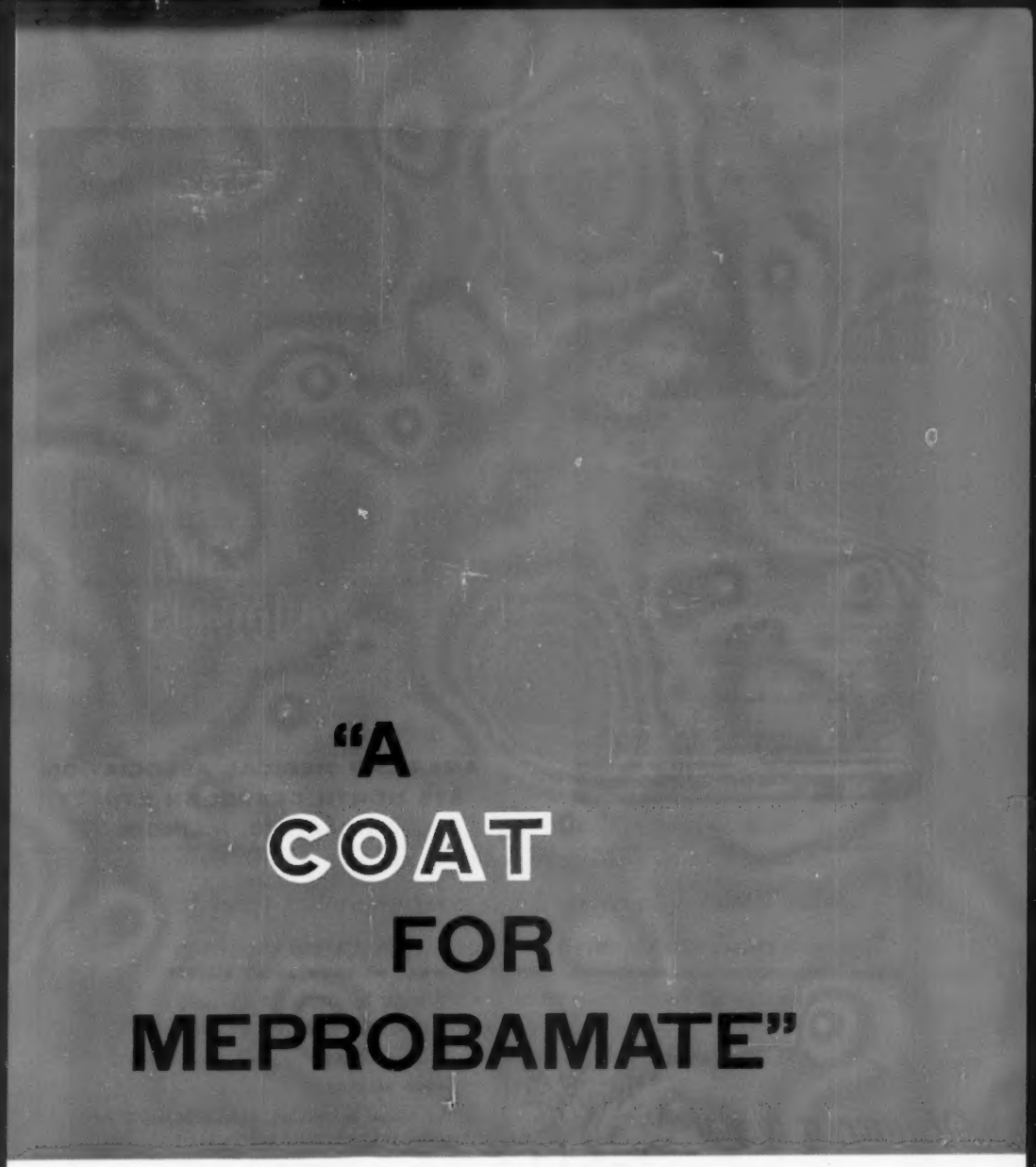
The Congress of the International Society of Blood Transfusions will be held at the same Palazzo dei Congressi on Sept. 3 to 6.

American Cancer Society Fellowships.—The American Cancer Society has announced that Clinical Fellowships at the senior resident level for the academic year 1959-1960 may be applied for by institutions accredited by the Council on Medical Education and Hospitals of the American Medical Association to give training in the following specialties and subspecialties, with emphasis on the diagnosis and treatment of cancer: internal medicine, malignant diseases, neurological surgery, obstetrics-gynecology, orthopedic surgery, otolaryngology, pathology, public health, radiology, surgery, and urology.

Institutions will be notified of awards granted in June, 1958. Individual candidates should apply directly to an institution or to the American Cancer Society for information concerning fellowships. The annual stipend, tax exempt, is $3600.

Application forms are available from the Director of Professional Education, American Cancer Society, Inc., 521 W. 57th St., New York 19.

Feb. 15, 1958, is the deadline for institutions submitting applications for the 1959-1960 clinical fellowships.



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Shwachman, H. and Leubner, H., Advances in Pediatrics, VII, 249, 1955.

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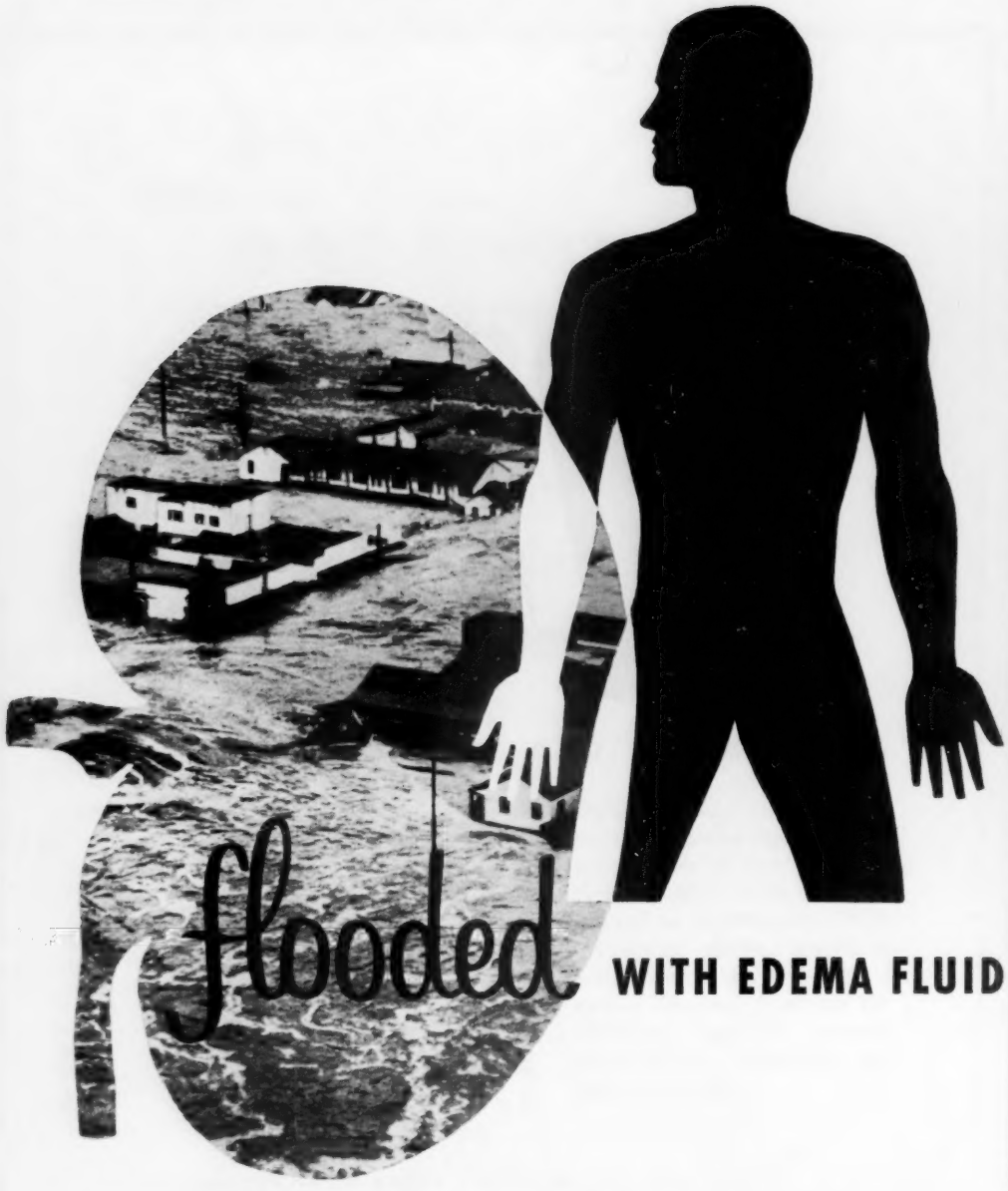

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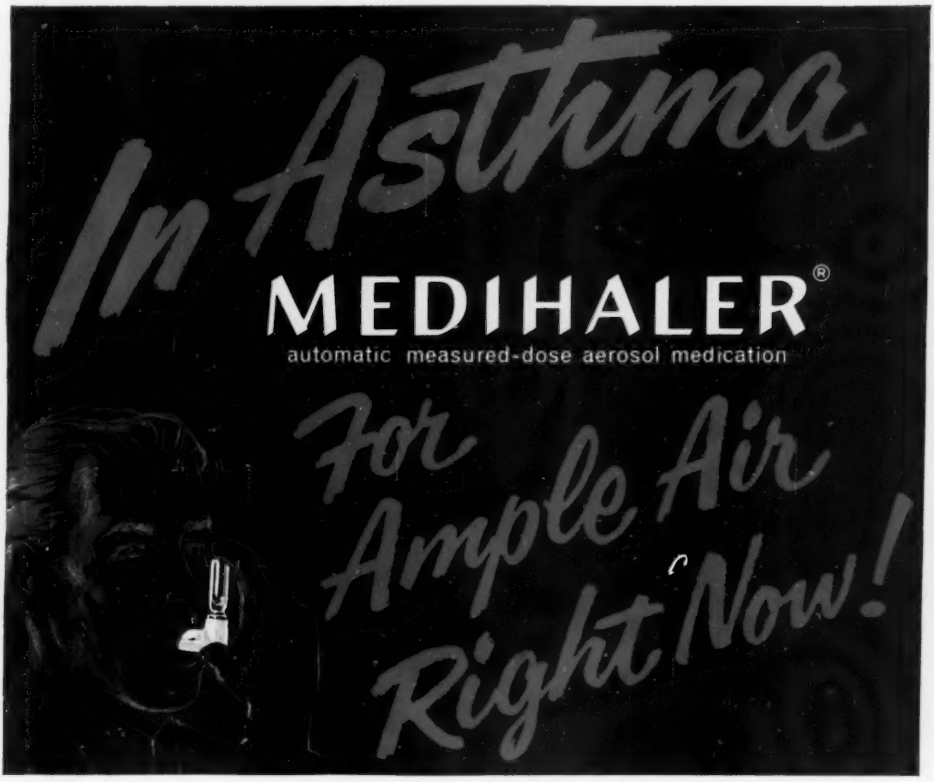

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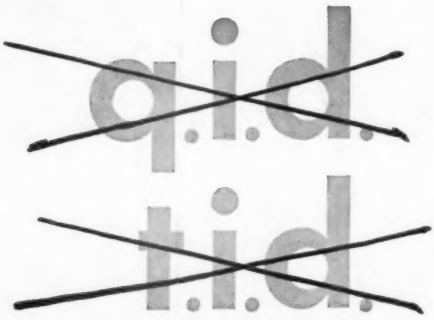
Riker LOS ANGELES

To simplify your management of ulcer and other g.i. disorders



new **Darbid*** Tablets, 5 mg.

b. i. d.

". . . a potent, long-acting anticholinergic drug requiring administration only twice daily"—Cayer[1]

CAYER and associates[1] reported 90% good to excellent results with 'Darbid' in 50 peptic ulcer patients with symptoms of nine years' average duration.

The authors made the following observations:

- Relief of ulcer pain after 'Darbid' therapy was "rapid and gratifying."
- ". . . the incidence of side effects was negligible."
- " 'Darbid' seems of particular value . . . because its long action simplifies administration."
- ". . . patients receiving 'Darbid' obtained significantly better results than those receiving atropine. . . ."

Dosage in this study was 5 mg. or 10 mg. orally, b.i.d.

Average duration of treatment: 8 months.

1. Cayer, D.; Sohmer, M.F., and Sugg, W.C.: The Effect of Prolonged Continuous Therapy on the Course of Chronic, Recurring Peptic Ulcer; Anticholinergic Therapy with SKF-4740 ('Darbid'), North Carolina M.J. *18*:311 (Aug.) 1957.

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